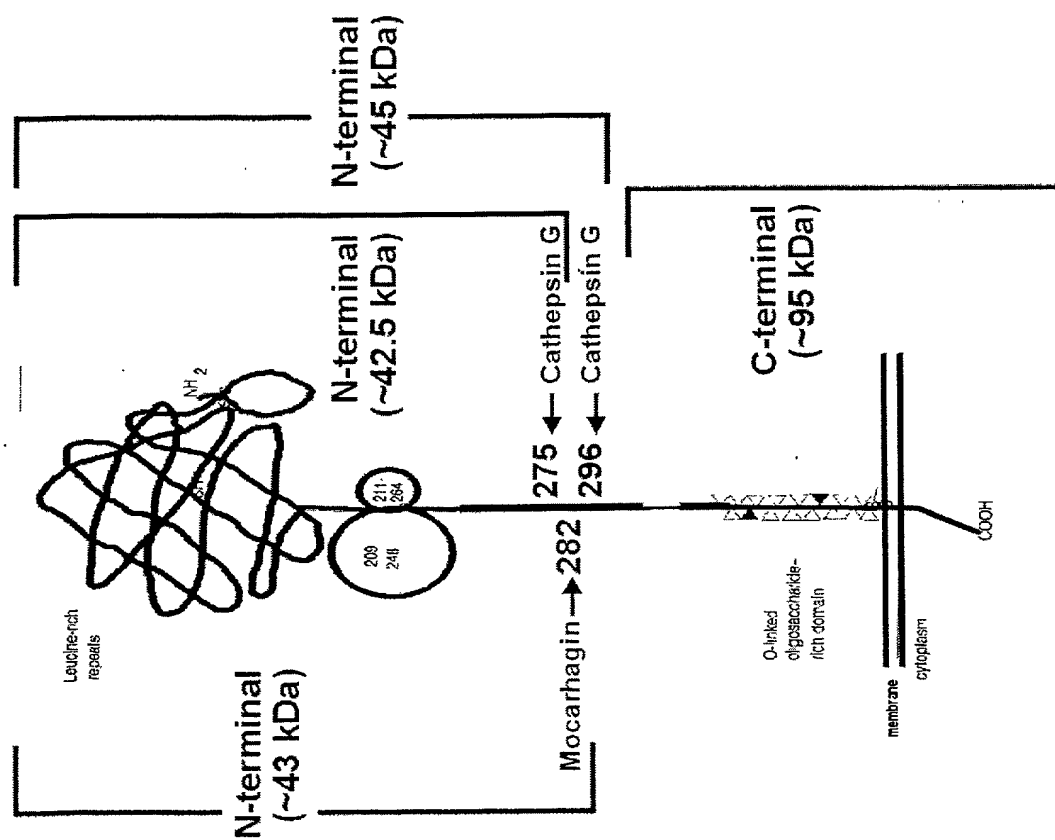


# Cleavage sites of endoproteases on the a-chain of GPIb

FIG. 1



# Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions

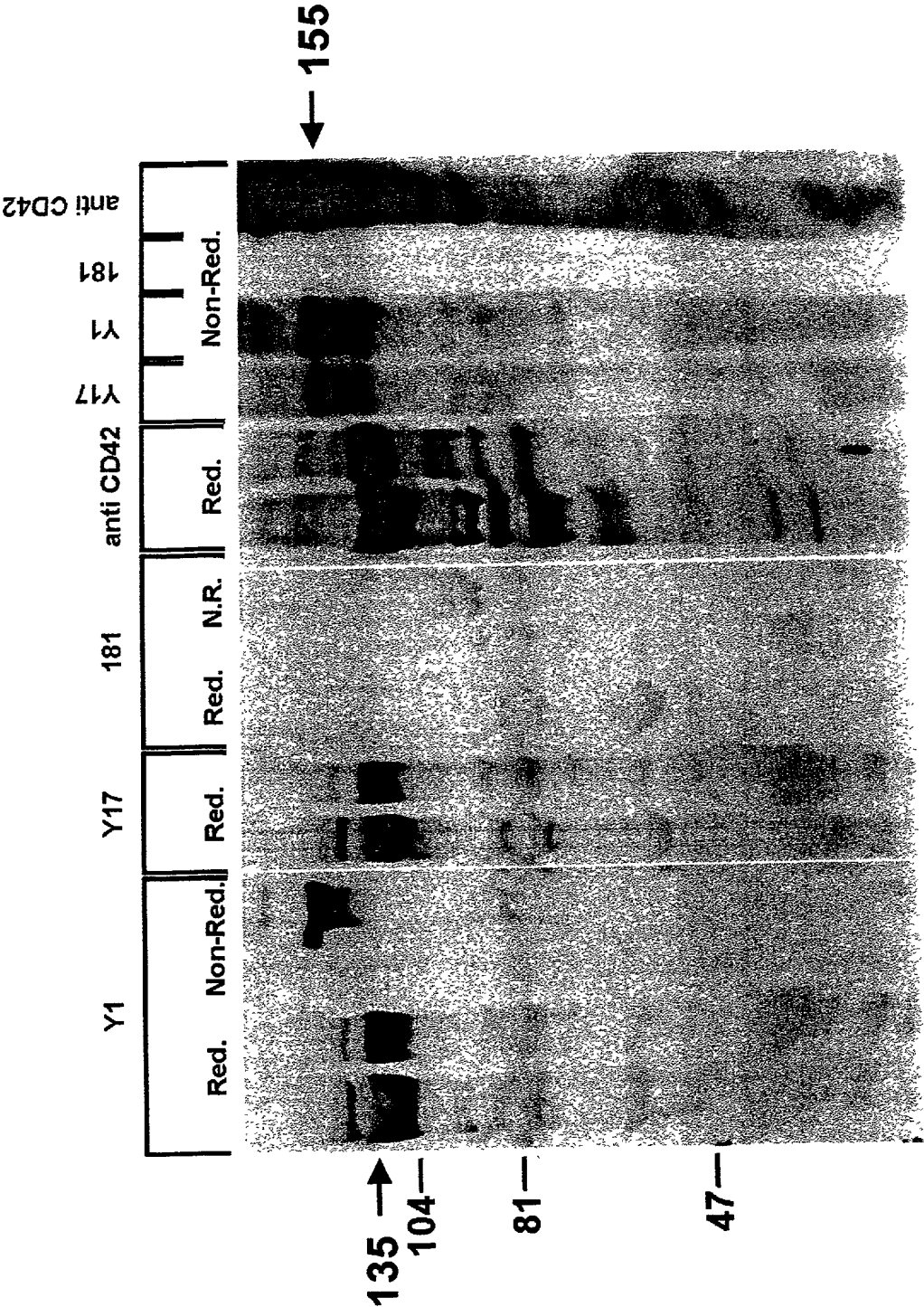


FIG. 2

**FIG. 3**

# **Characterization of Optimal Determinants for Binding of Y1 to It's Ligands**

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+

# Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17

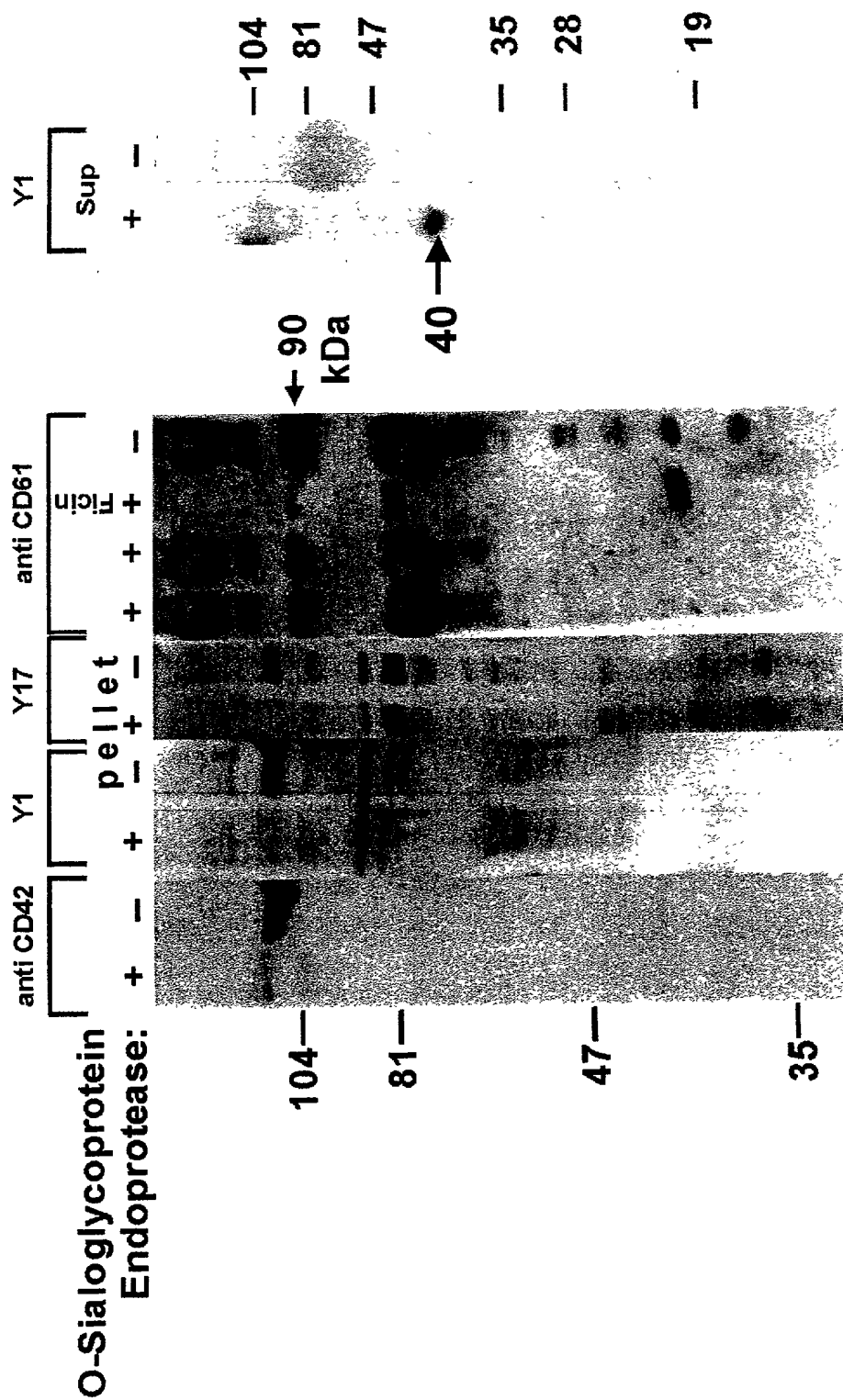


FIG. 4

Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease

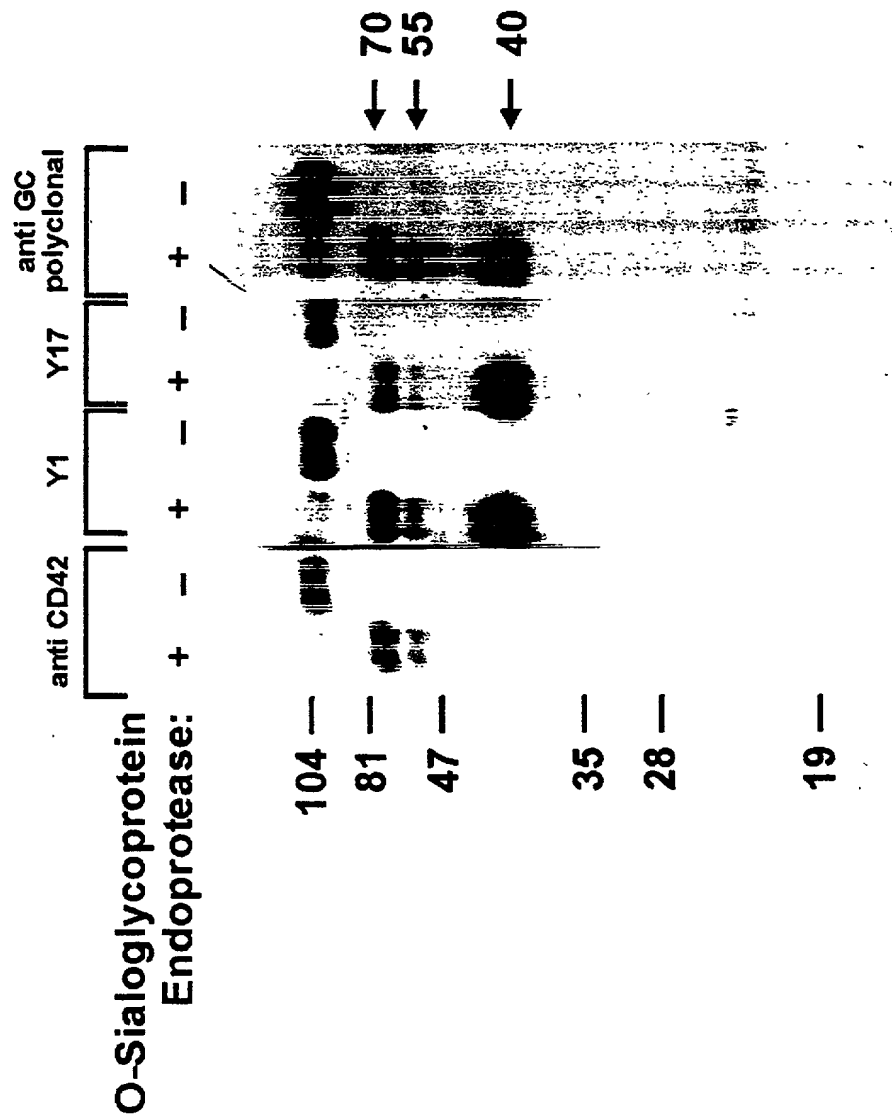
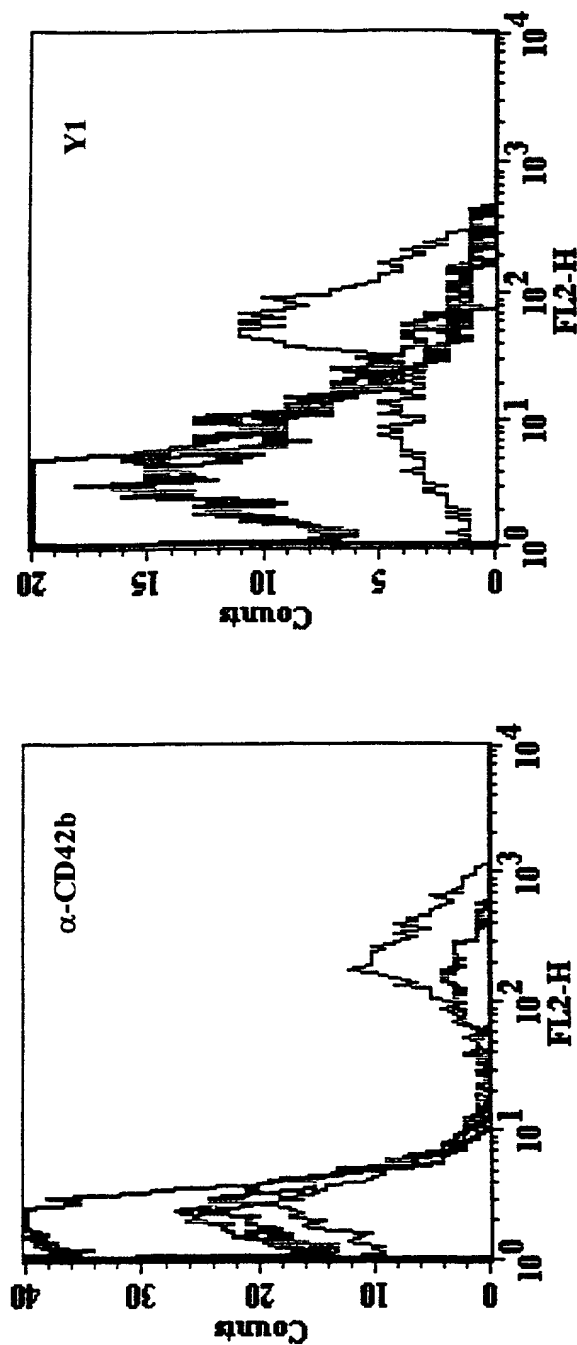


FIG. 5

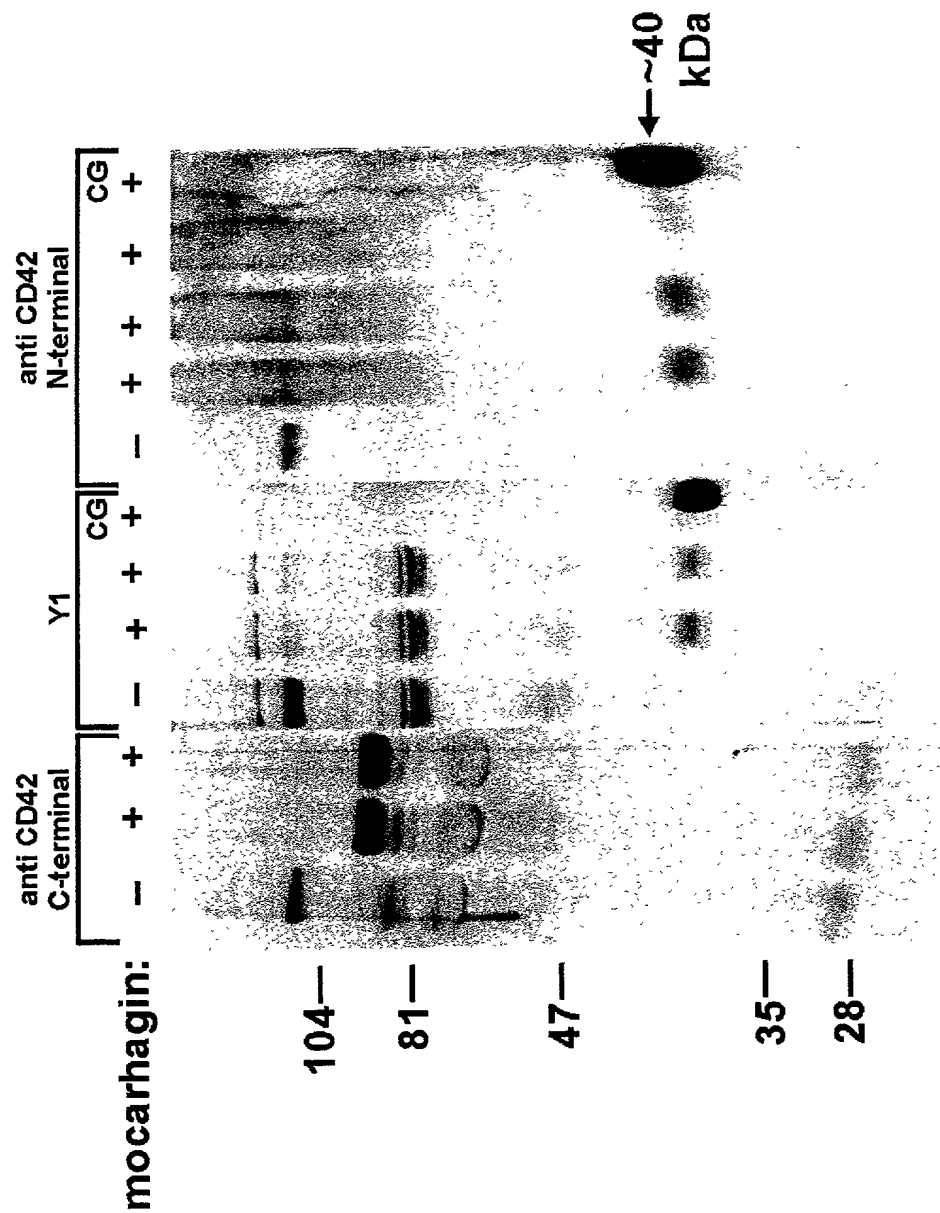
# Specific GPIIb Proteolysis Abolishes Y1 Binding to Platelets



Key	Name	Parameter	G
	NON-TREATED PLATELETS		
—	O-SIALOGLYCOPROTEIN ENDO. (10 $\mu$ g/ml)		
- -	O-SIALOGLYCOPROTEIN ENDO. (50 $\mu$ g/ml)		
— · —	FICIN (18 $\mu$ g/ml)		

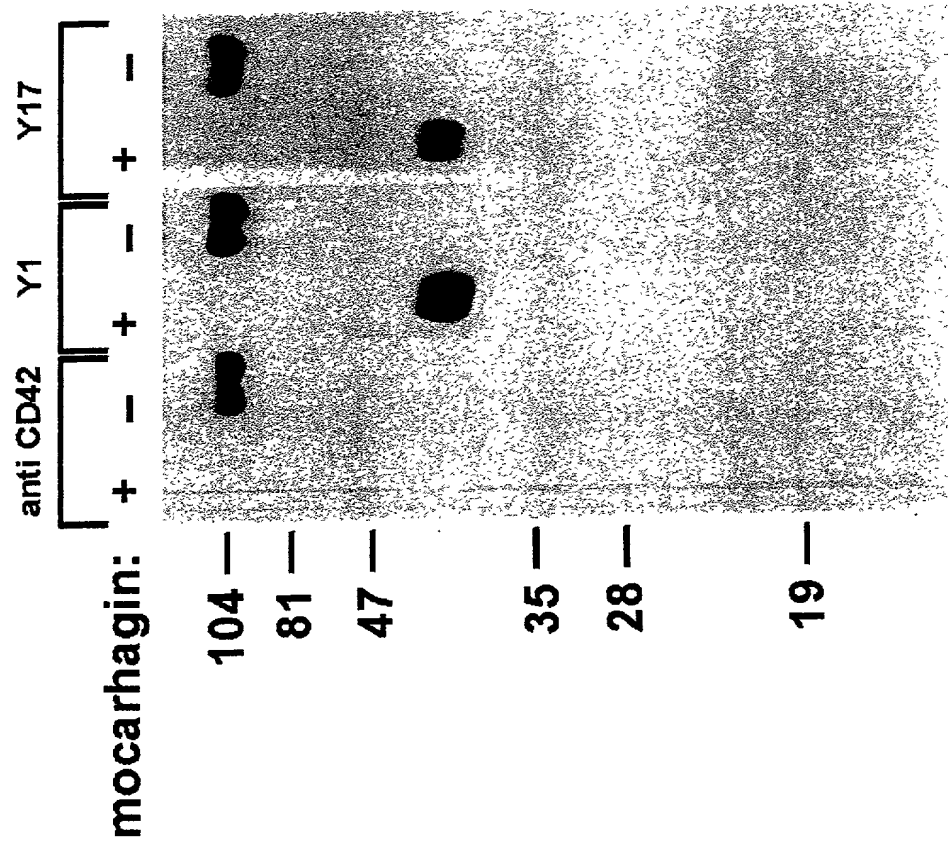
FIG. 6

**Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin**



**FIG. 8**

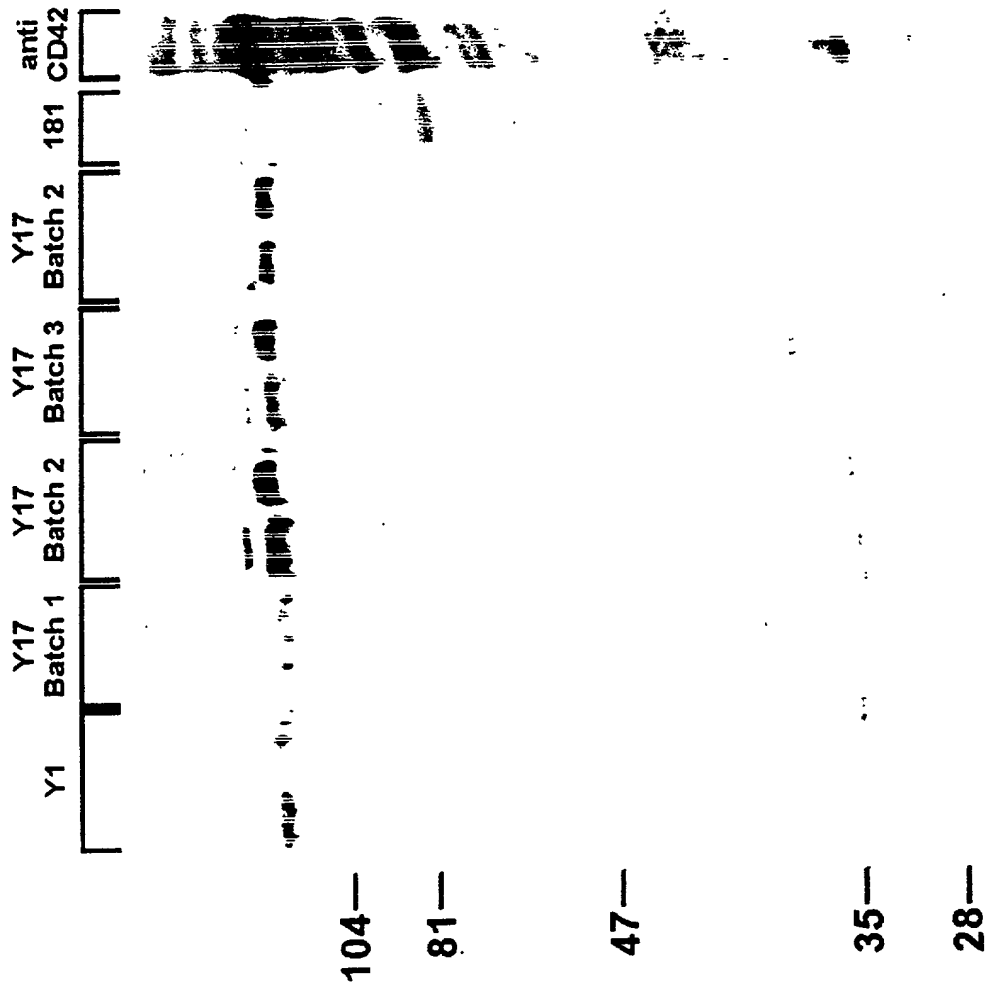
## Binding of Y1 and Y17 to glycolalycin after cleavage by mocarhagin





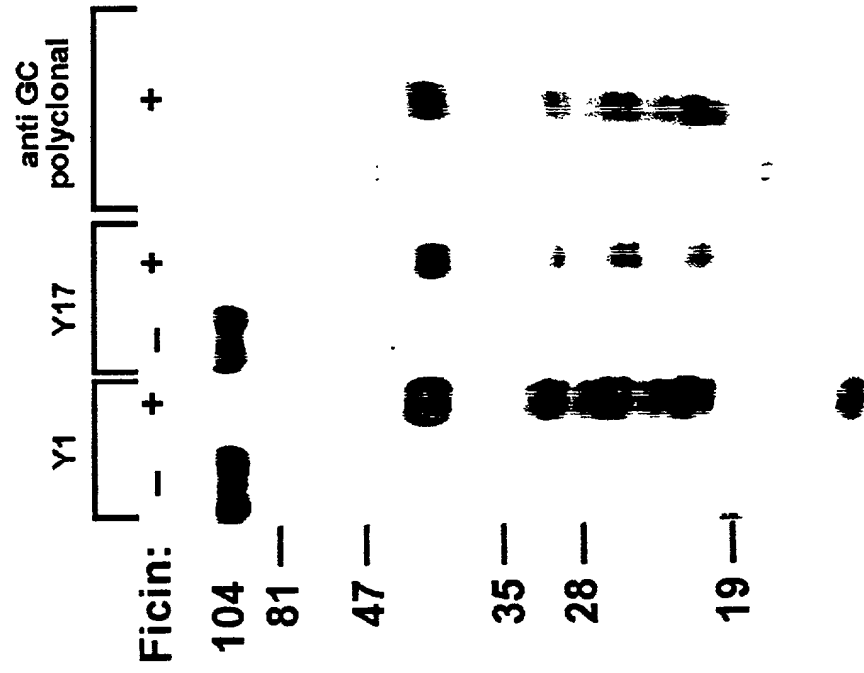
**FIG. 9**

# Binding of Y1 and Y17 to platelets

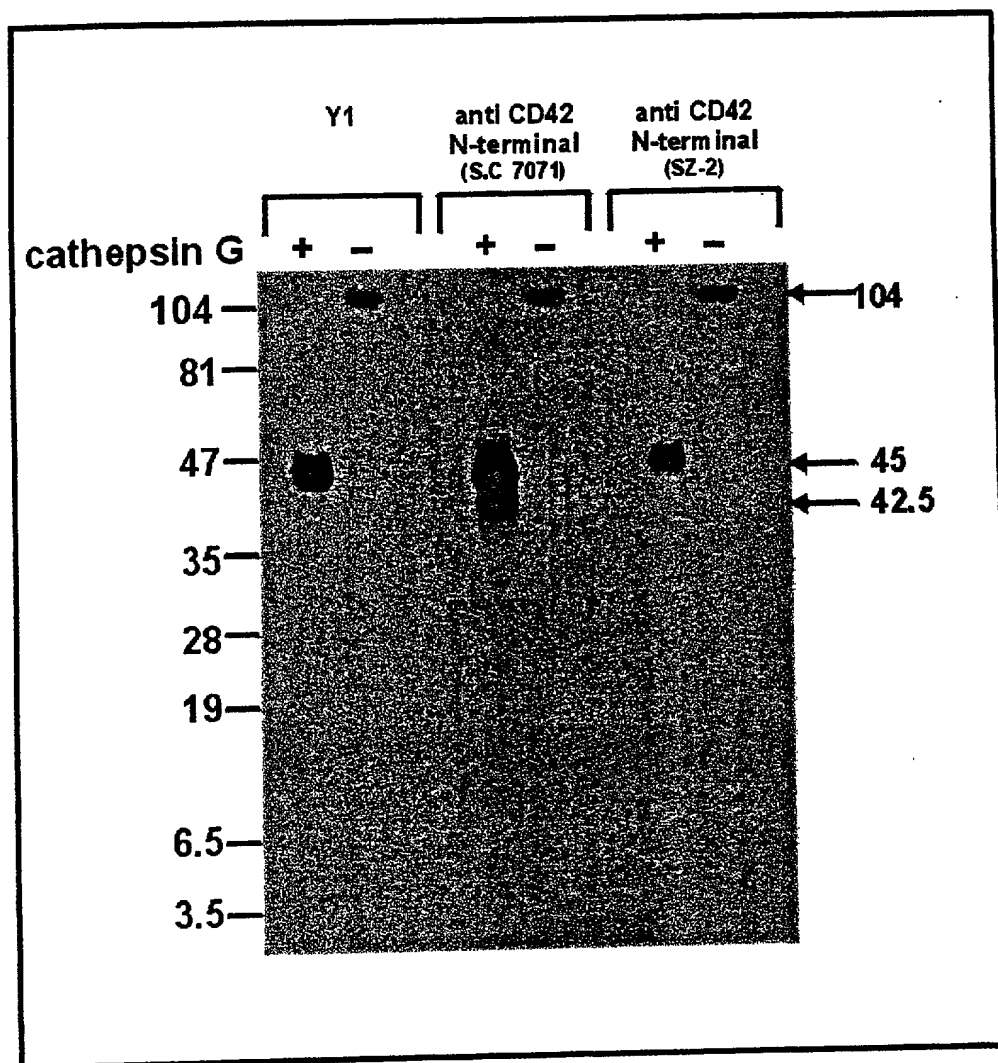


**FIG. 10**

Y1 and Y17 bind glycosylcalycin similar after cleavage by Ficin



**FIG. 11**



# Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment

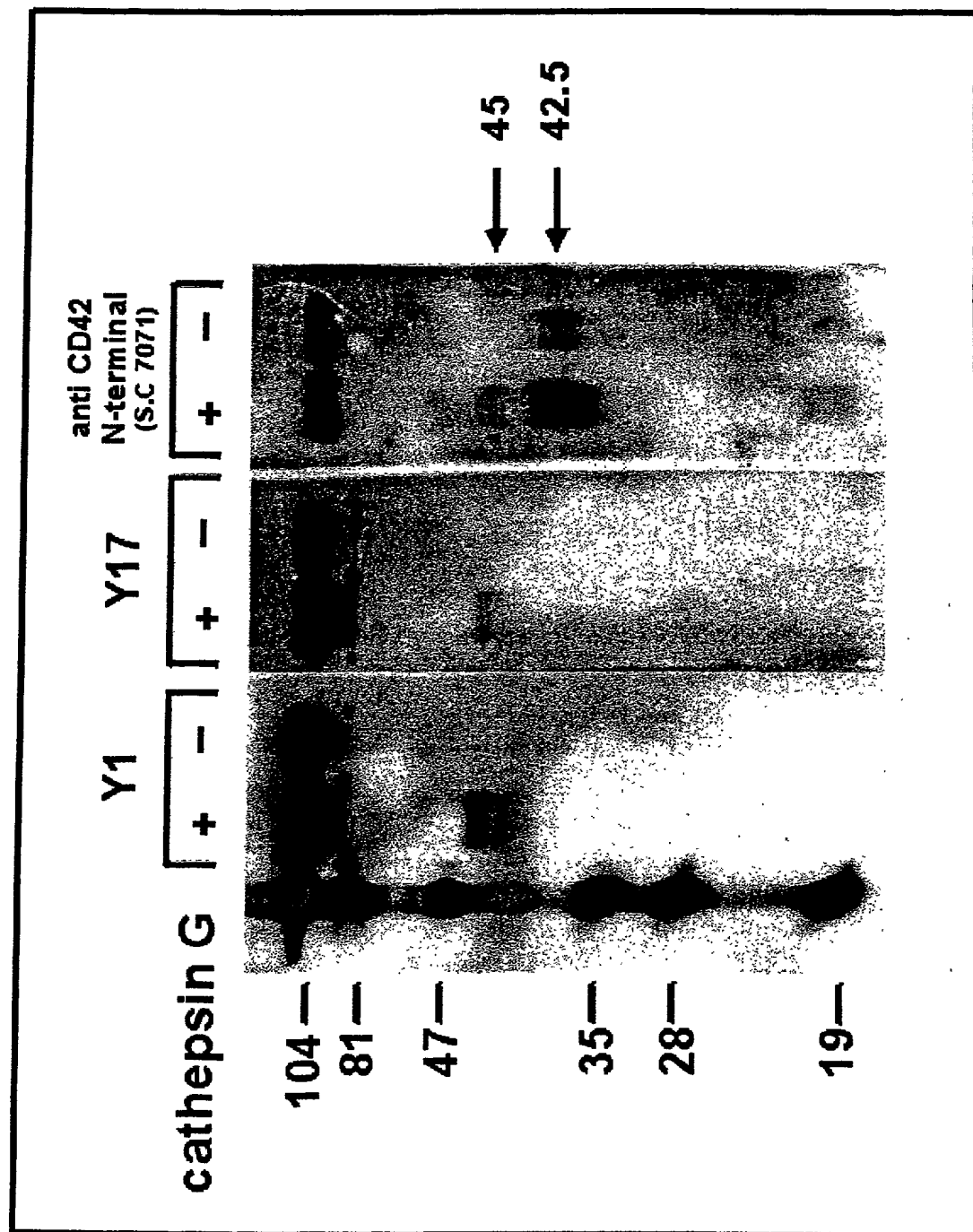
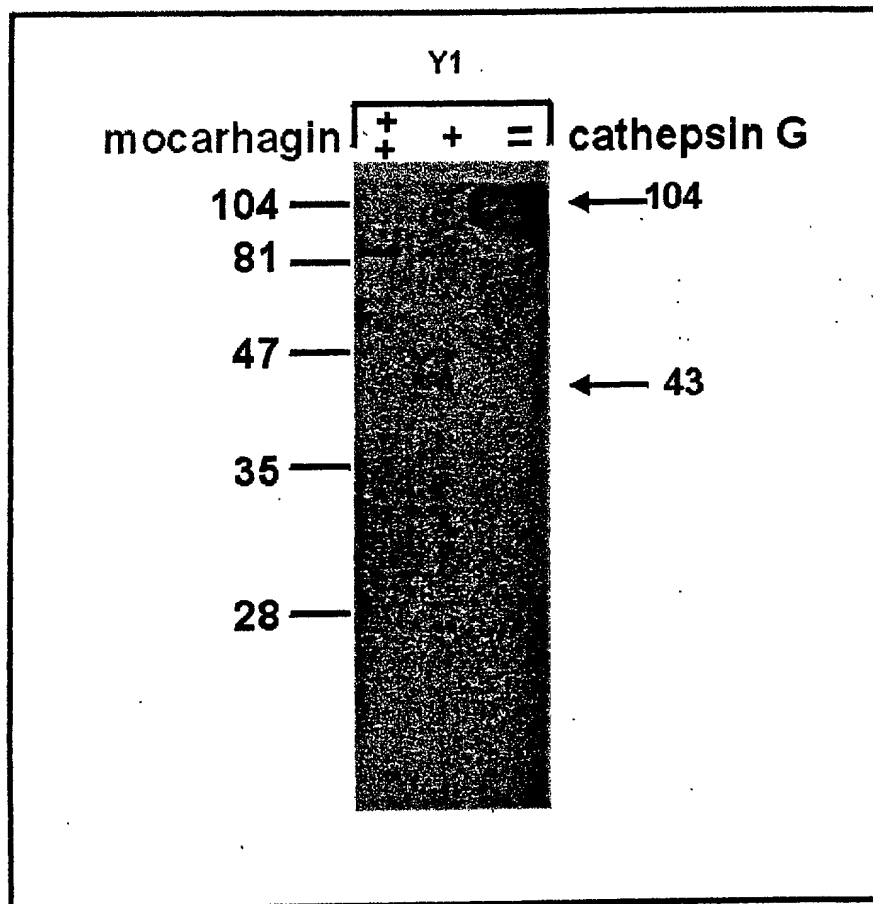
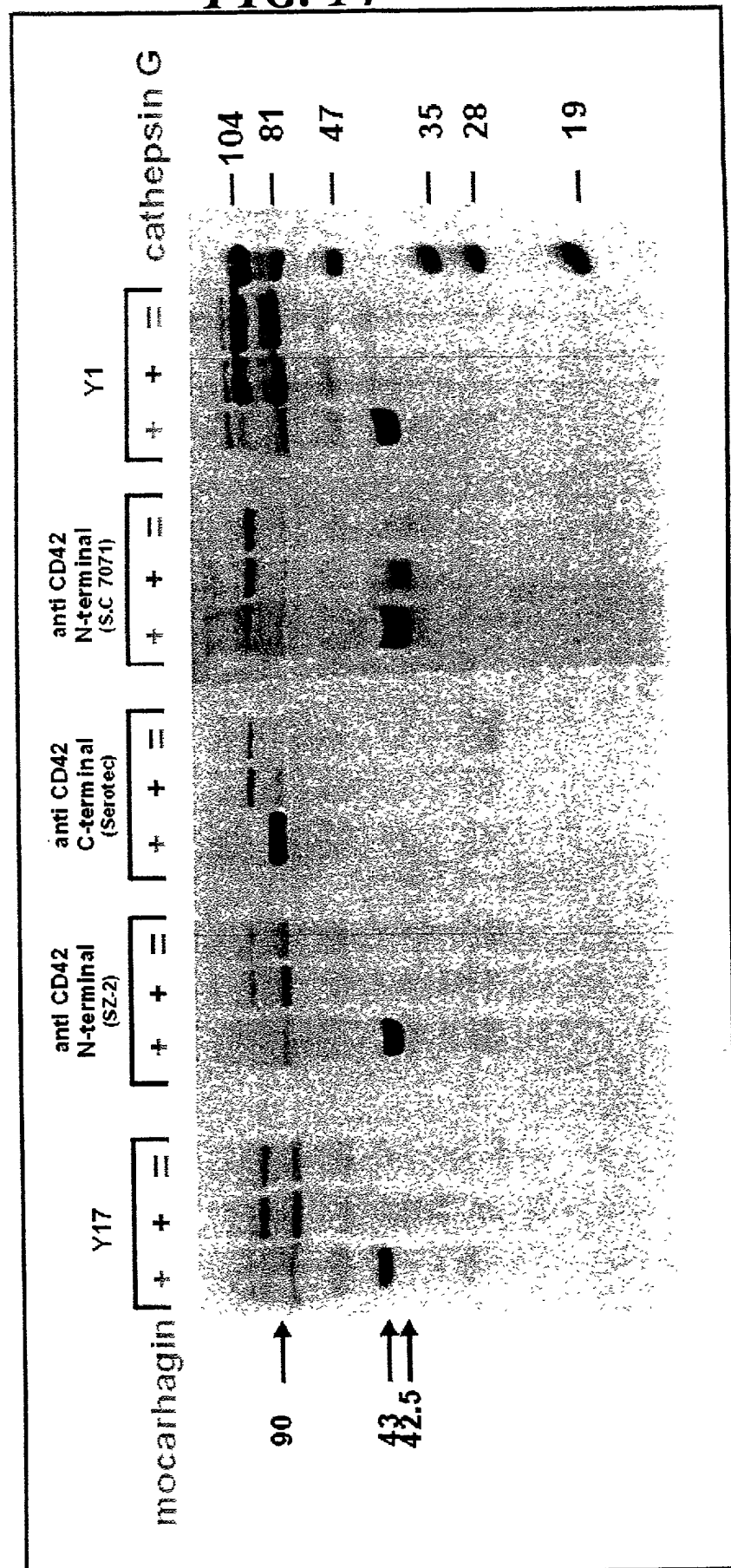


FIG. 12

**FIG. 13**

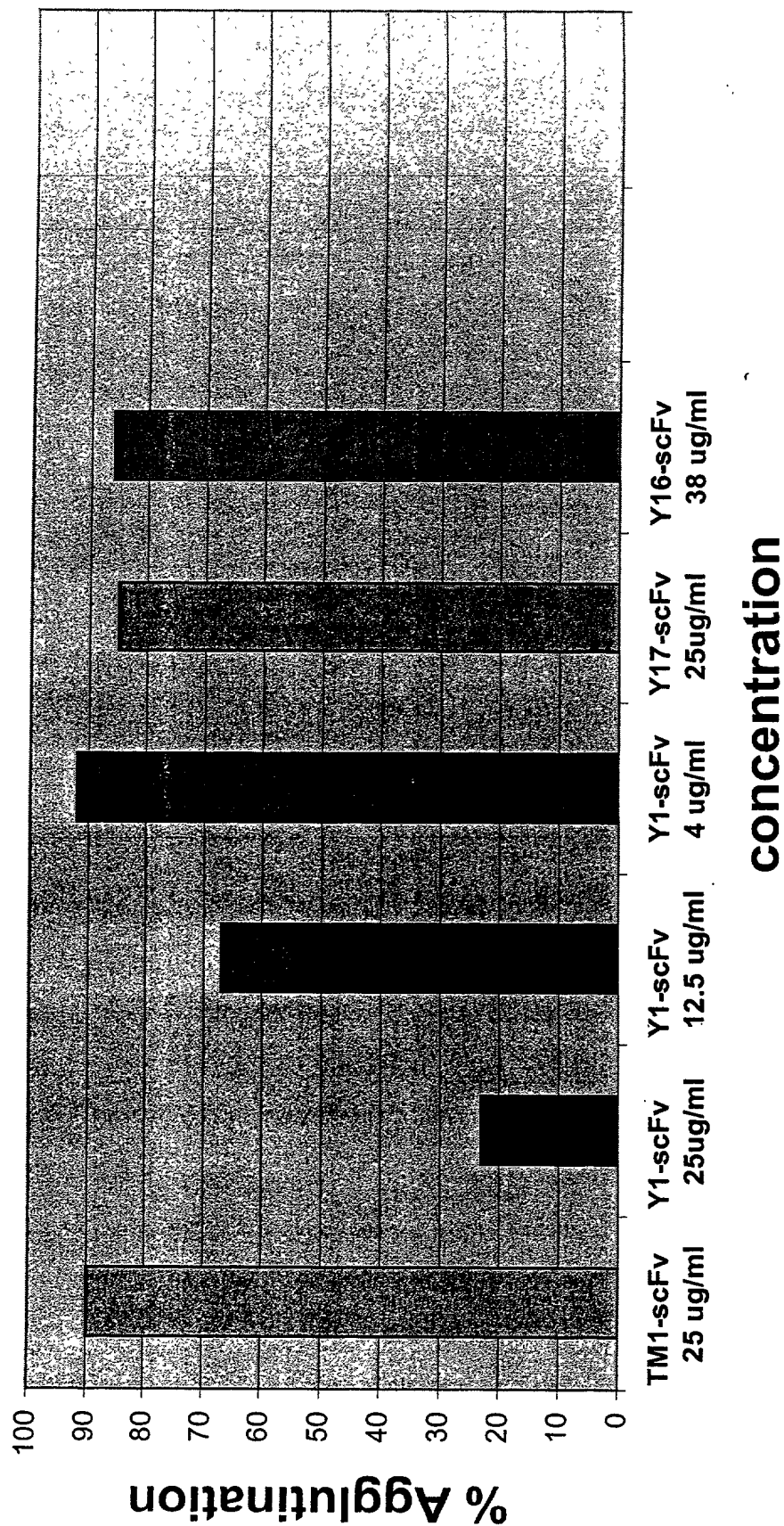


# Cleavage of washed platelets by mocarhagin and cathepsin G

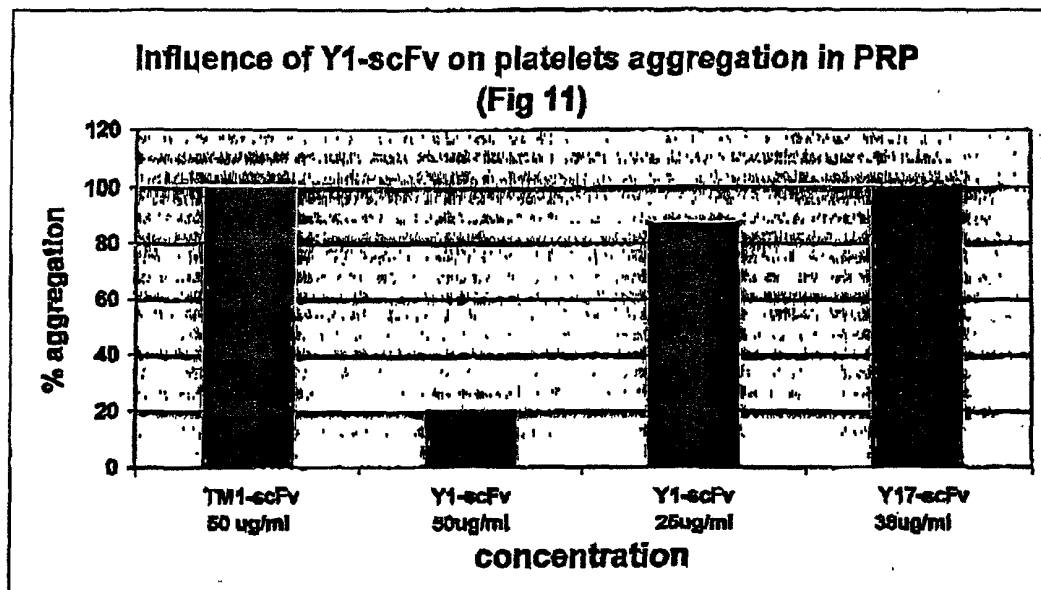


**FIG. 15**

**Influence of Y1-scFv on platelets agglutination in washed platelets**



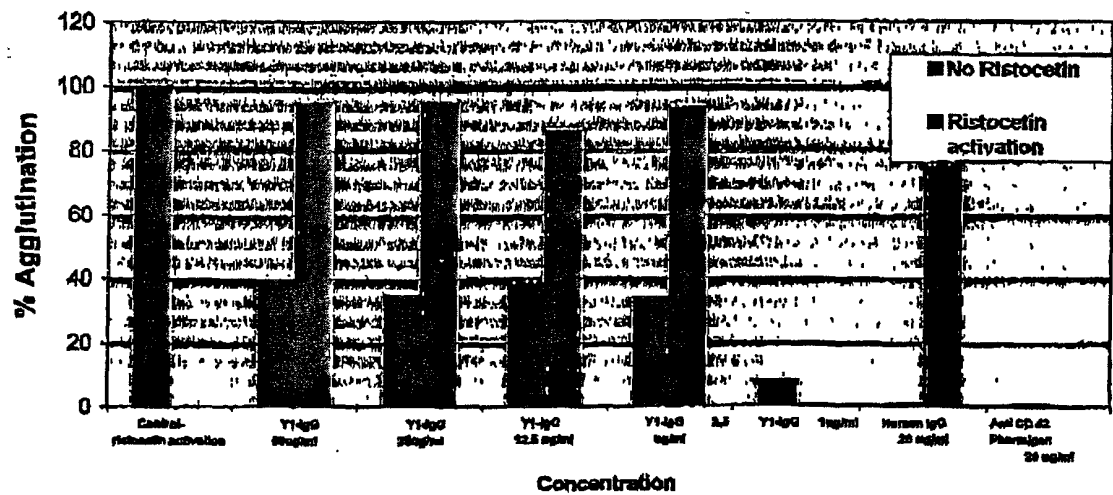
**FIG. 16**





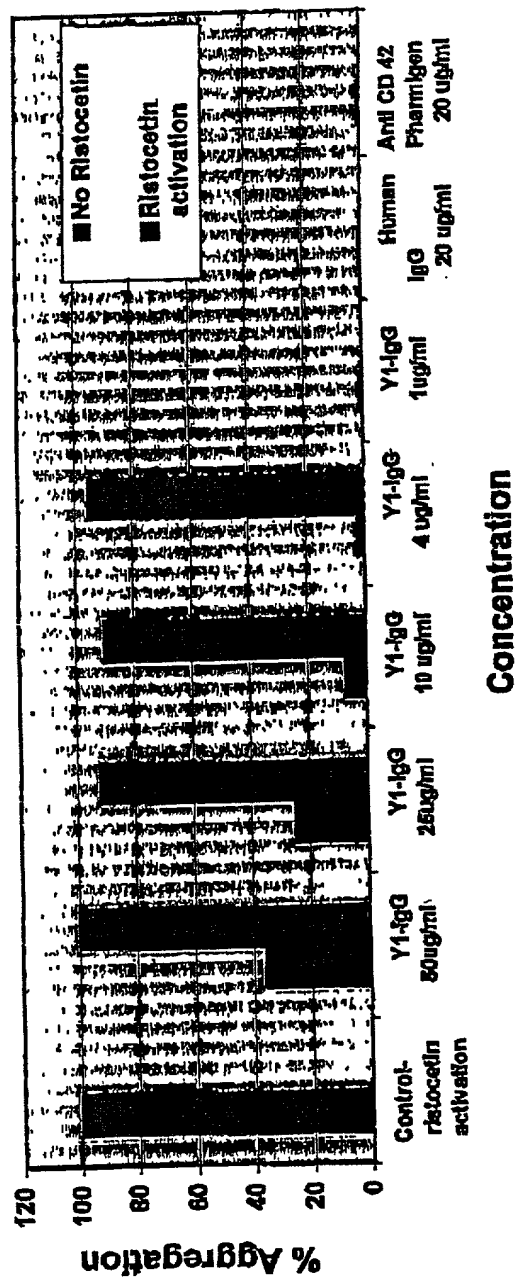
**FIG. 17**

**Induction of platelet agglutination by Y1-IgG in washed platelets**

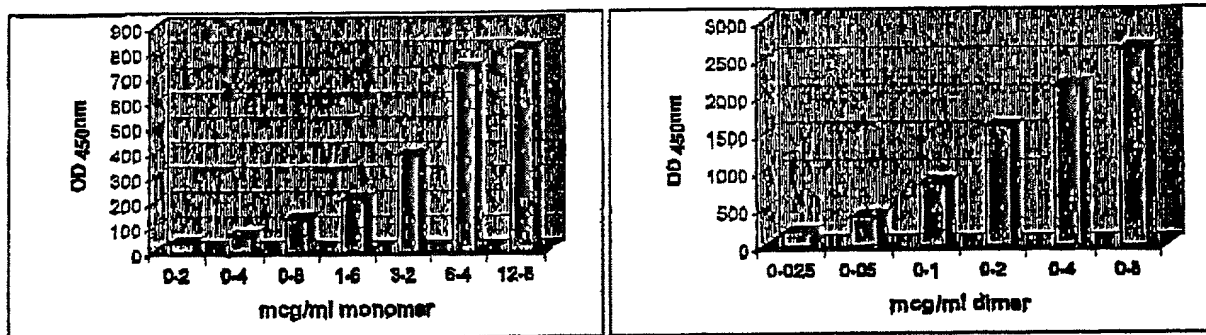


**FIG. 18**

**Induction of platelet aggregation by Y1-IgG in PRP**

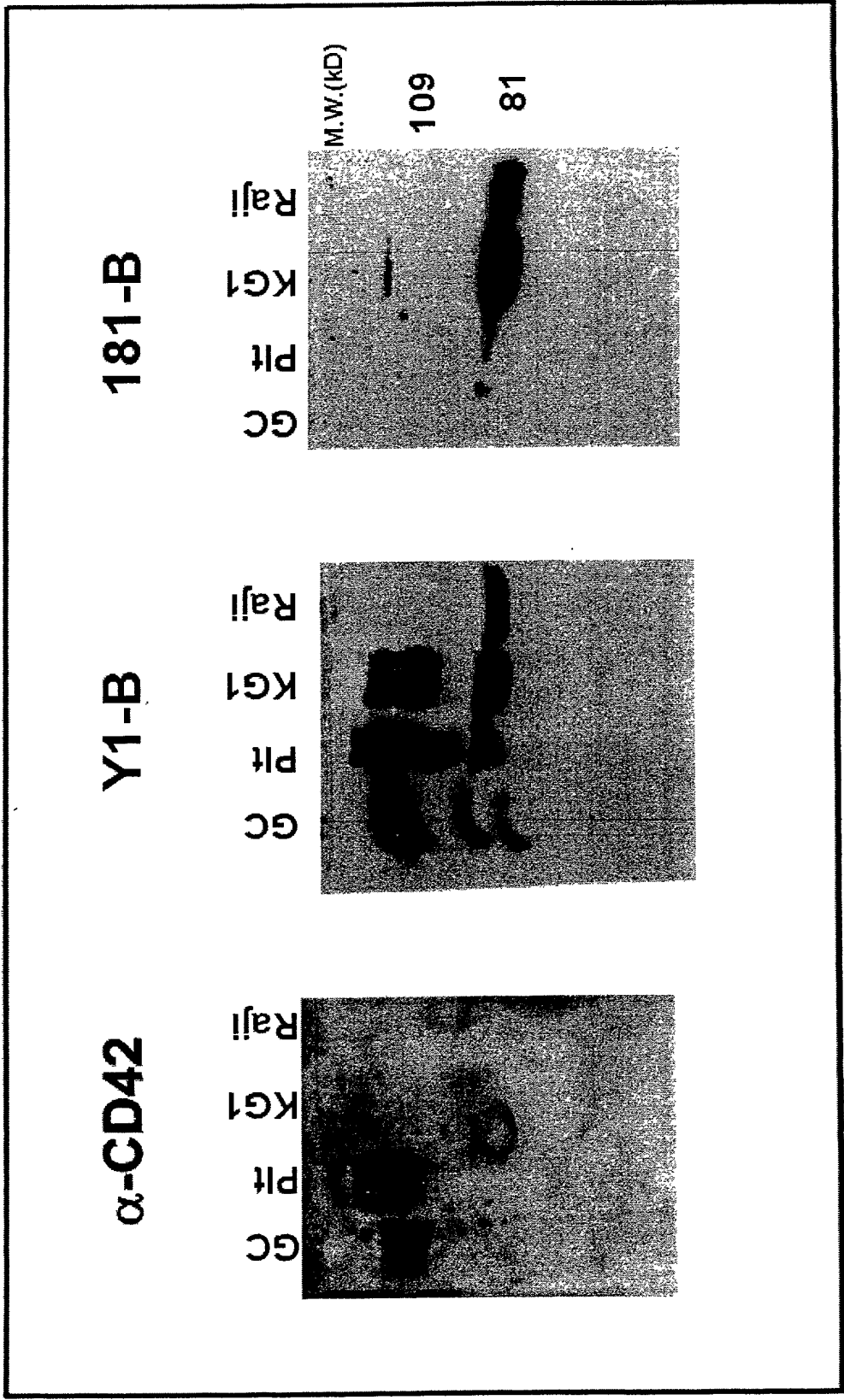


*FIG. 19*



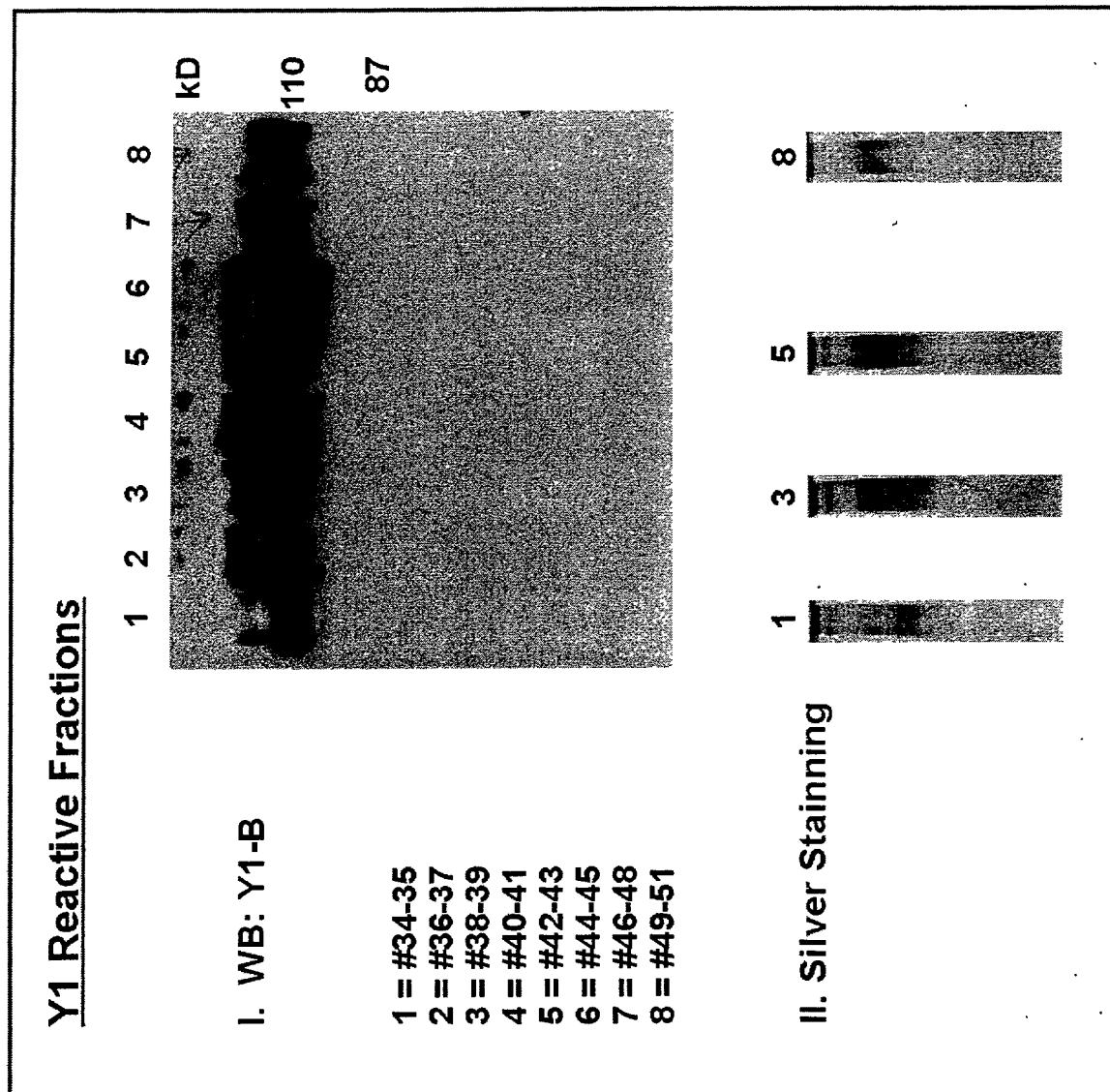
**FIG. 20**

**Specificity of Binding of Y1  
and  $\alpha$ -CD42 (N1-19)  
to their Ligands**



**FIG. 21**

# Y1-Ligand from KG1 membranes following Immuno-Precipitation with Y1: Purification on RP-HPLC



# Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

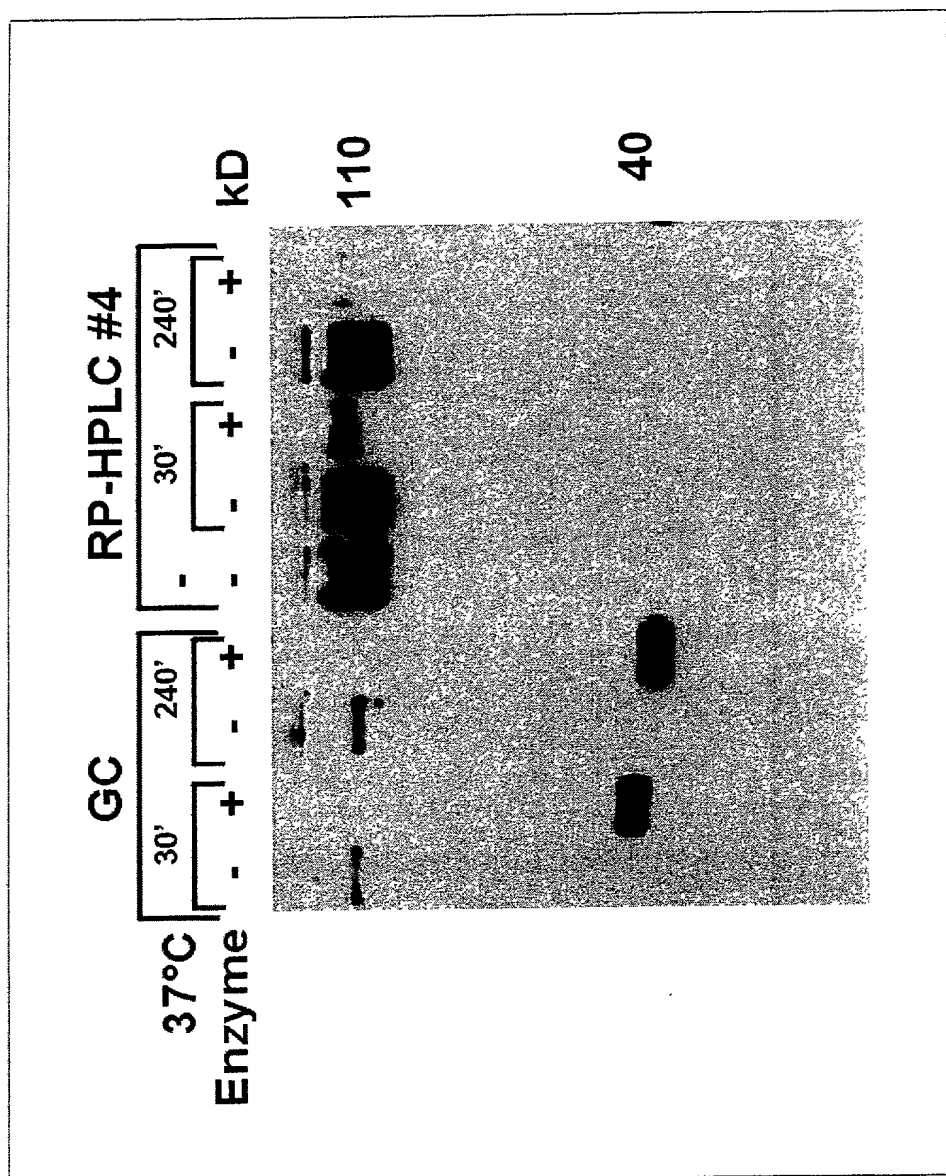
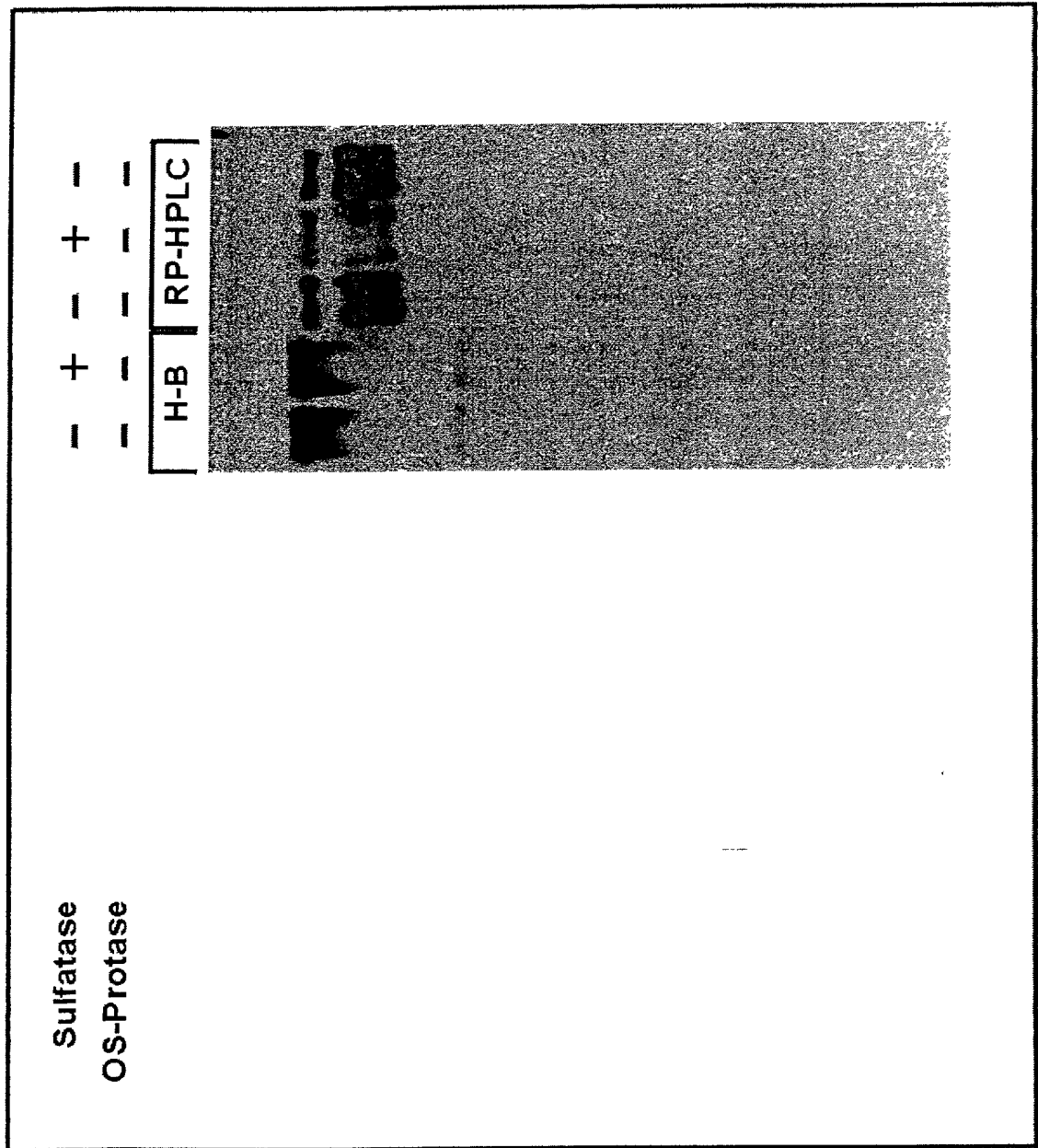


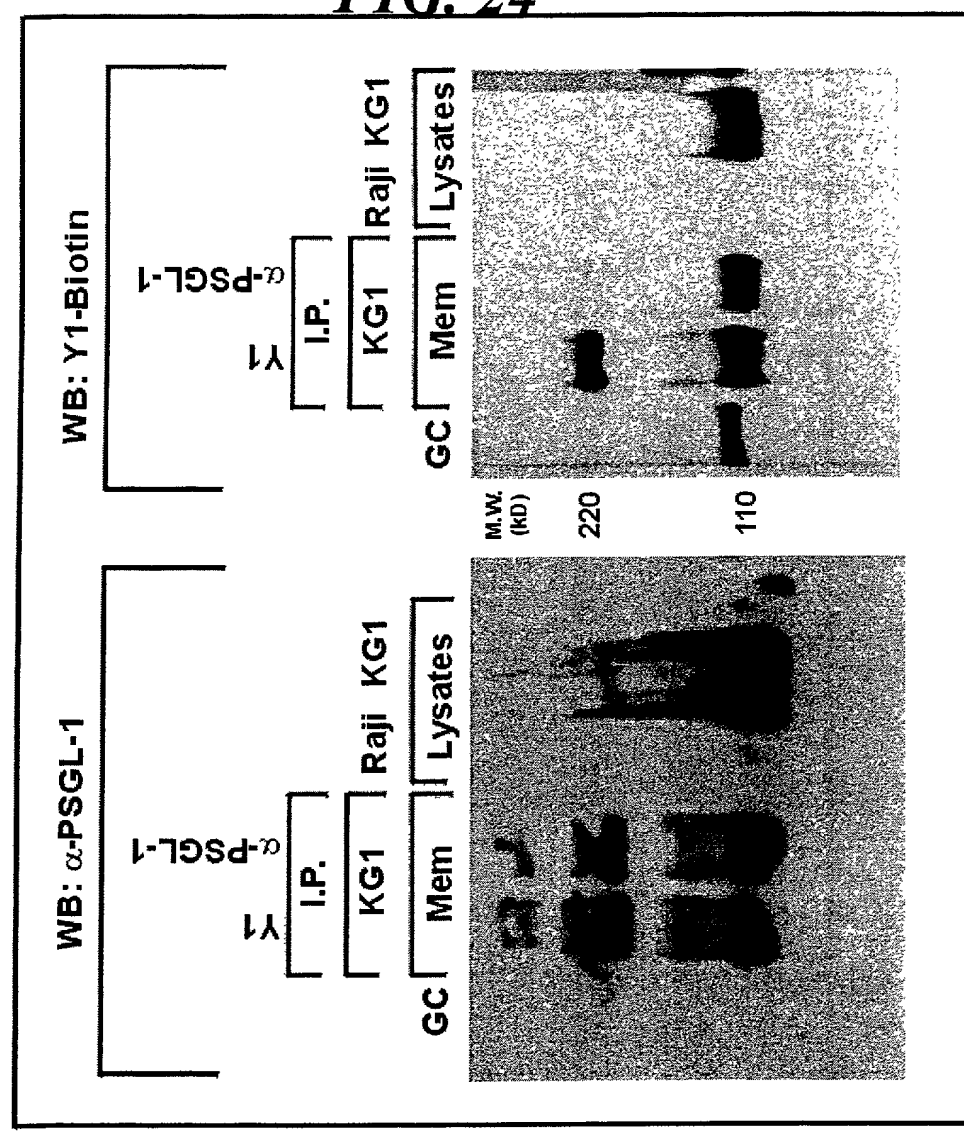
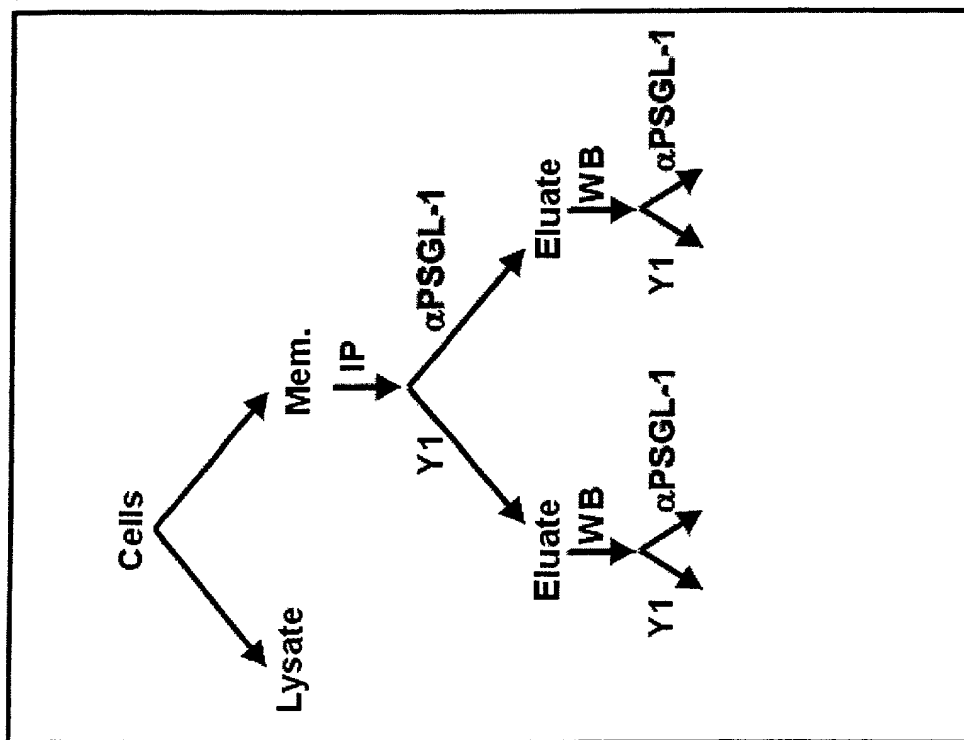
FIG. 22

# Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

FIG. 23



# Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

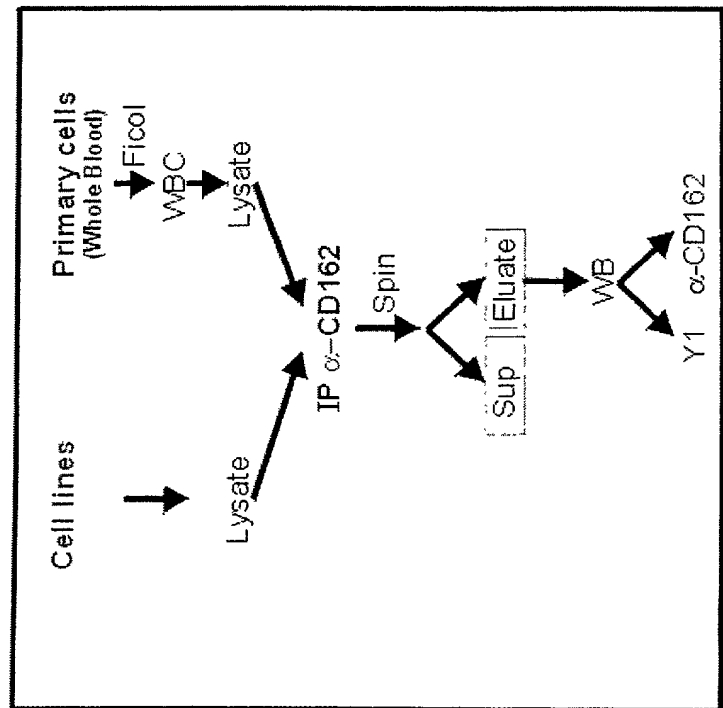


**FIG. 24**



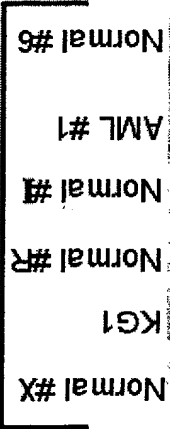
**FIG. 25**

$\alpha$ -CD162 and Y1:  
Comparison between cells  
from AML patient and normal  
blood

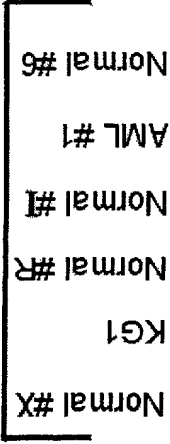


**I**

**WB:  $\alpha$ PSGL-1**

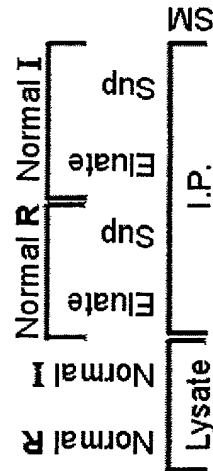


**WB: Y1-B**

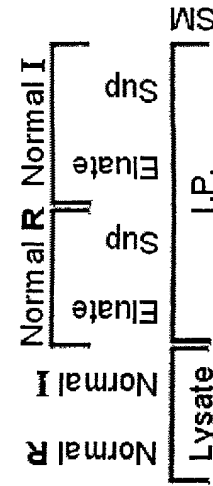


**II**

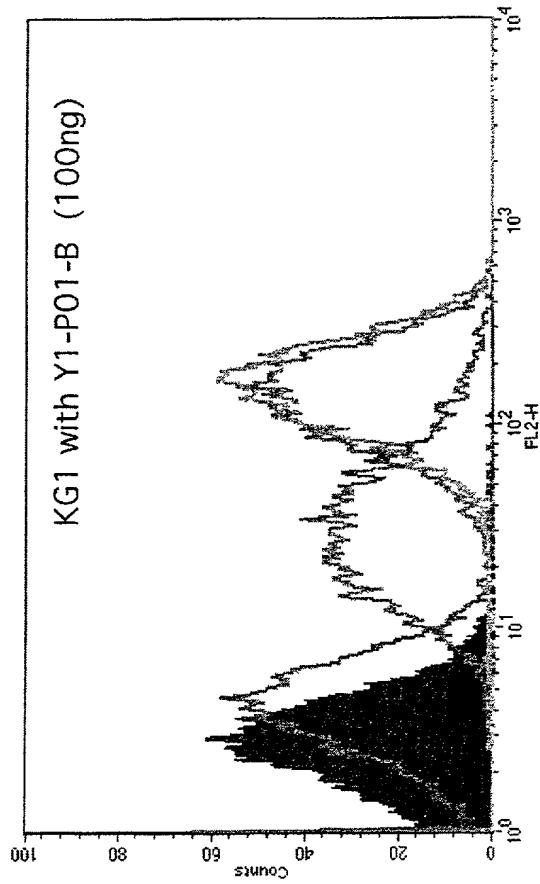
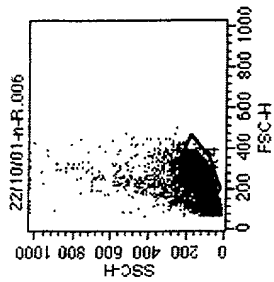
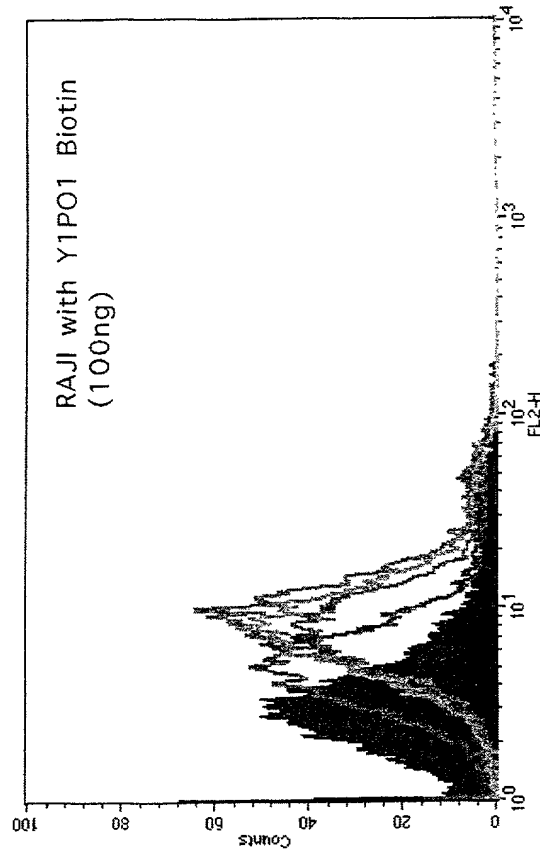
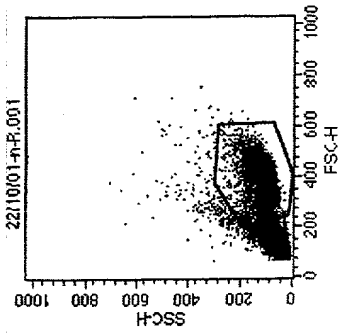
**WB:  $\alpha$ PSGL-1**



**WB: Y1-B**



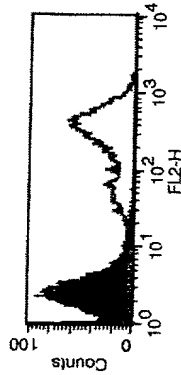
# FIG. 26



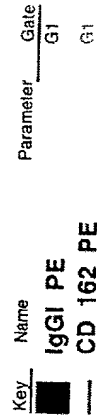
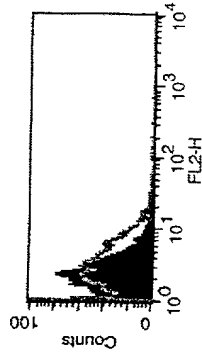
Key	Name	Parameter	Gate
	22/10/01-n-R.006	N01-B	
	22/10/01-n-R.006	P01-B	
	22/10/01-n-R.006	+KPL1	
	22/10/01-n-R.006	+PL1	
	22/10/01-n-R.006	+PL2	

# Specificity of Y1 Binding: Analysis by FACS

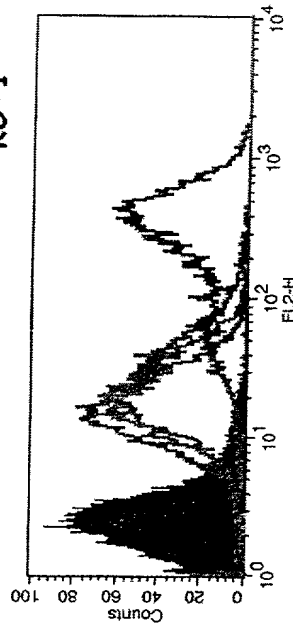
KG-1



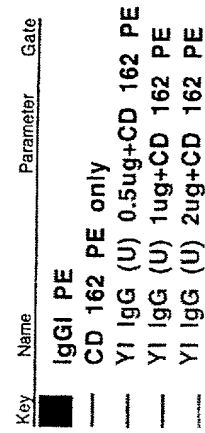
Raji



KG-1



CD 162 PE



- Binding of  
 $\alpha$  PSGL1  
( $\alpha$ CD162/KPL1);  
competition  
with Y1-IgG

FIG. 27

# Specificity of Y1 Binding: Analysis by FACS

- Binding of Y1-IgG; competition with  $\alpha$ PSGL-1 (CD162 /KPL1)

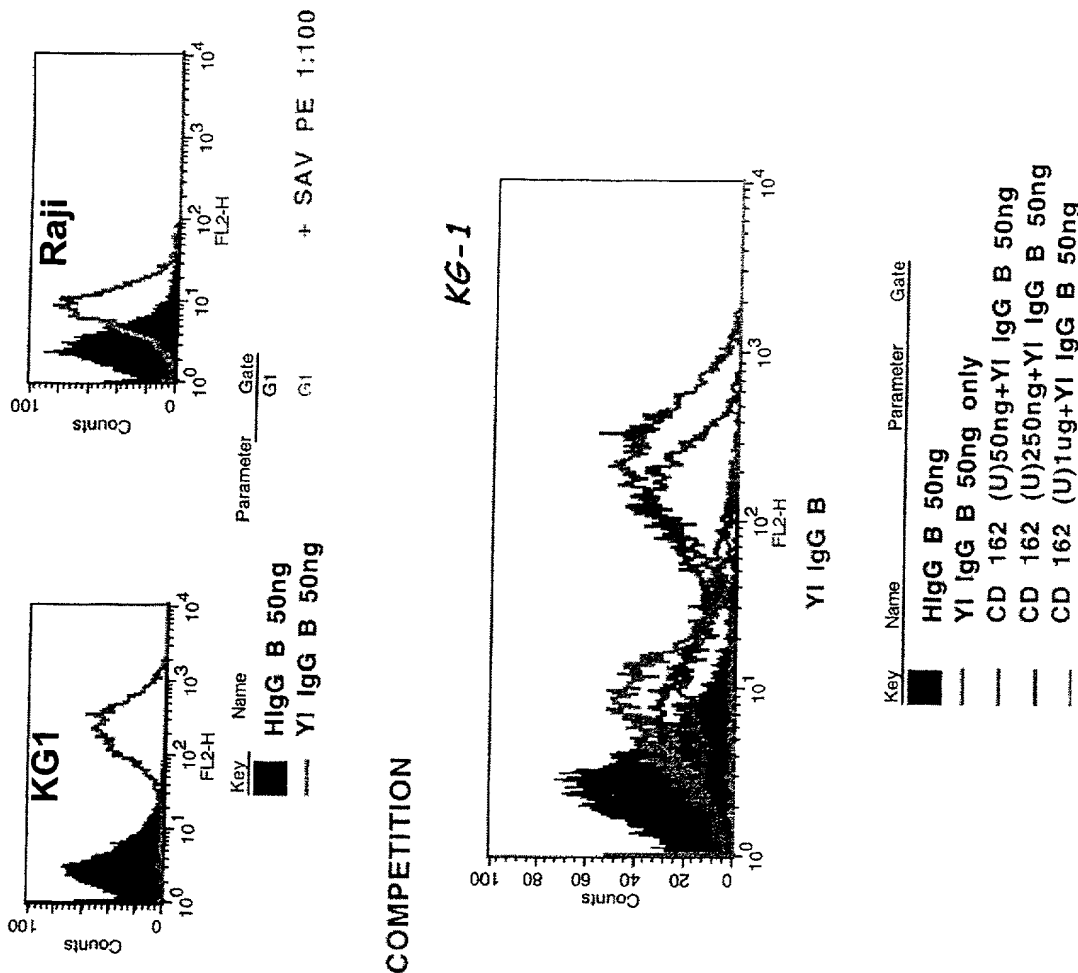
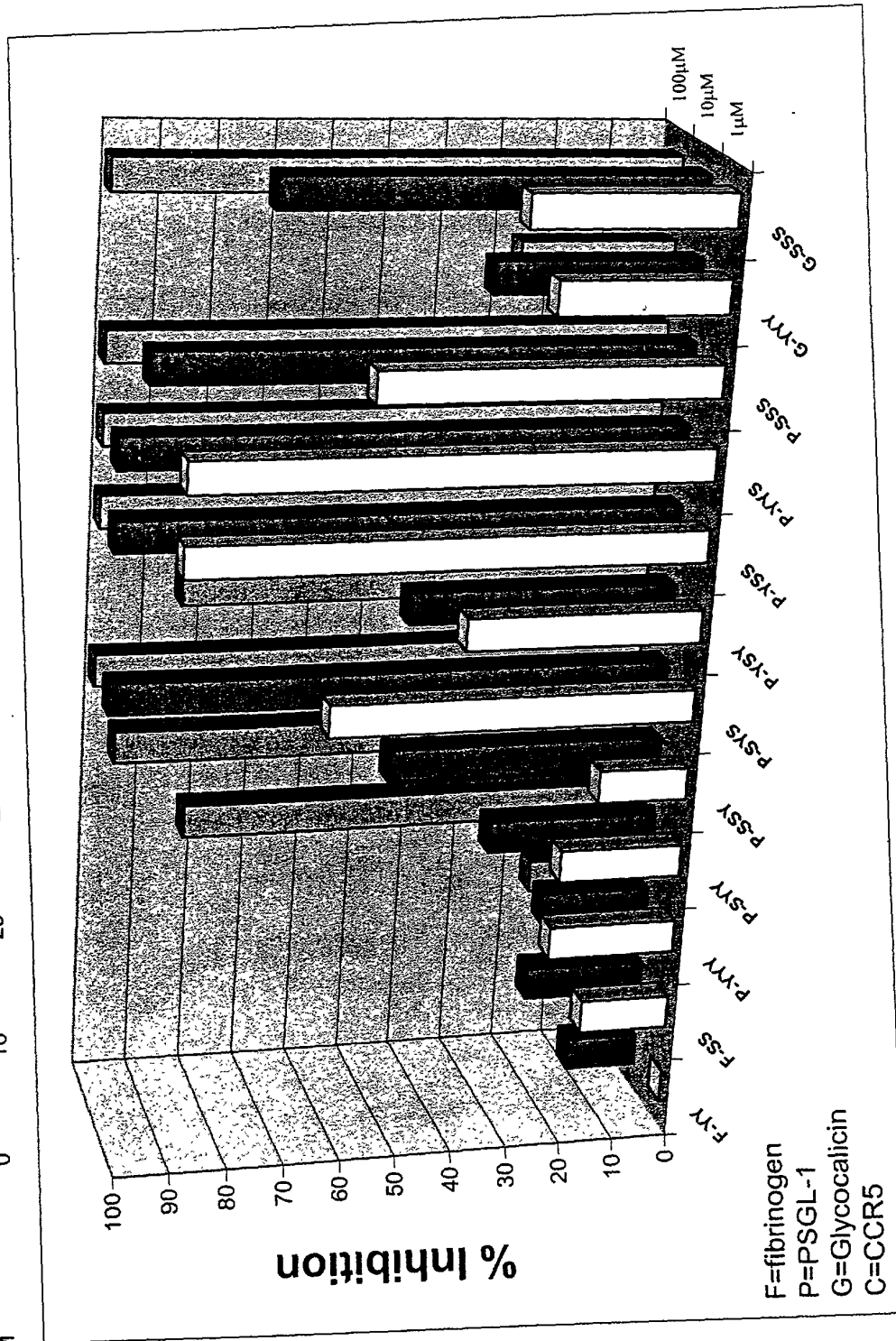


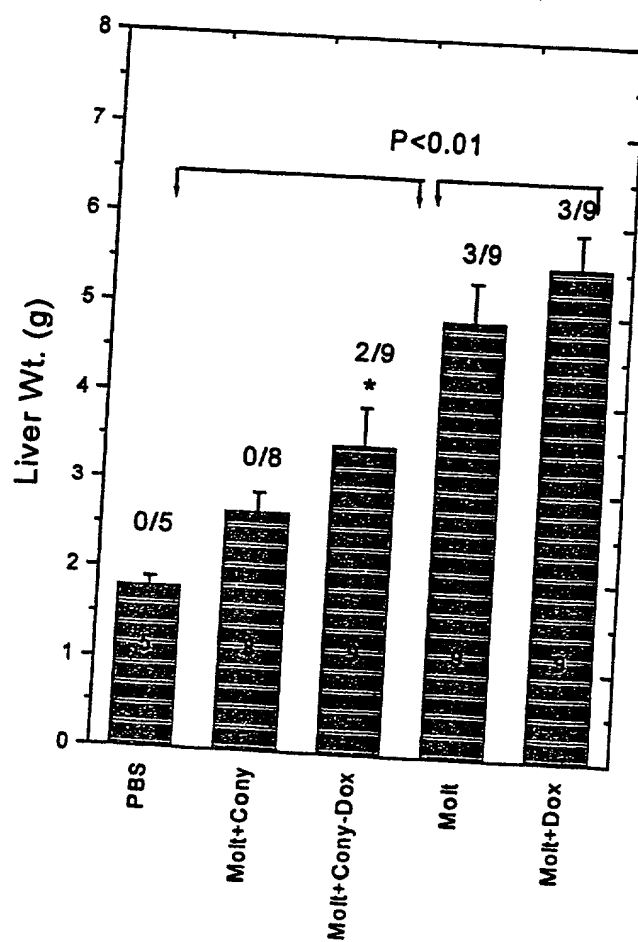
FIG. 28

# FIG. 29

	F-YY	F-SS	P-YYY	P-SYY	P-SSY	P-SYS	P-YSY	P-YSS	P-YYs	P-SSs	G-YYY	G-SSs
100µM	0	0	18	83	96	100	100	85	100	100	100	28
10µM	13	22	20	31	50	100	100	48	100	100	95	75
1µM	0	16	23	22	16	65	91	42	91	60	30	36

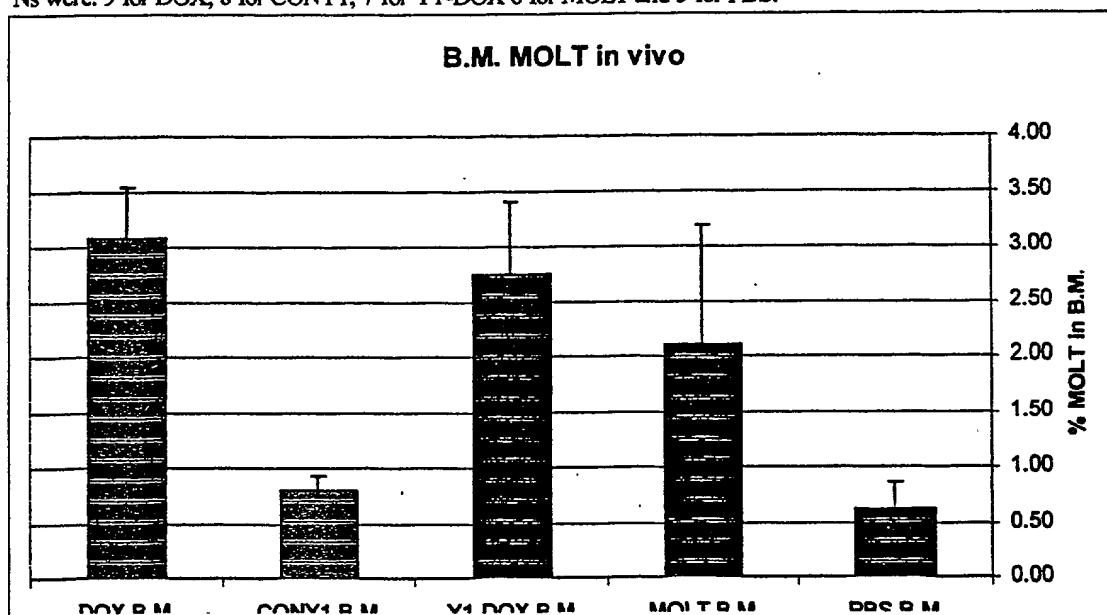


**FIG. 30**

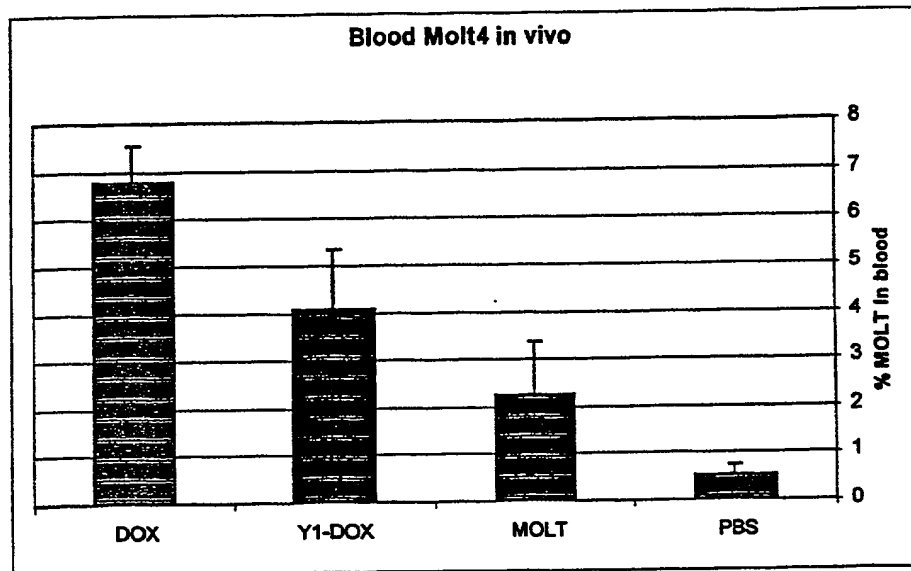


**FIG. 31**

\*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.



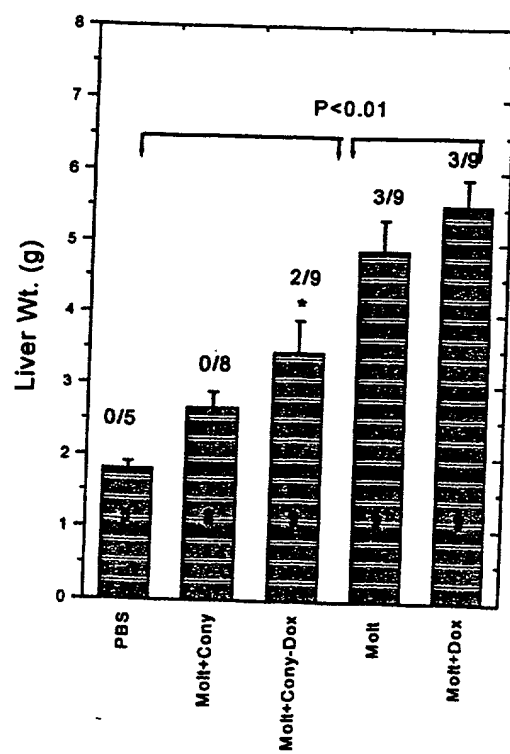
**FIG. 32**



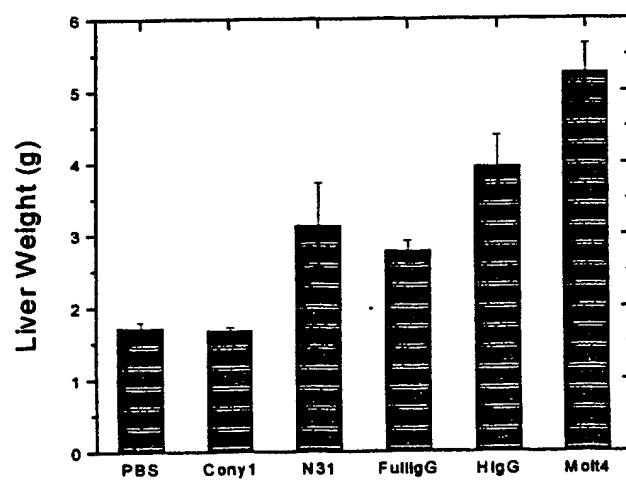
\*\*Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.



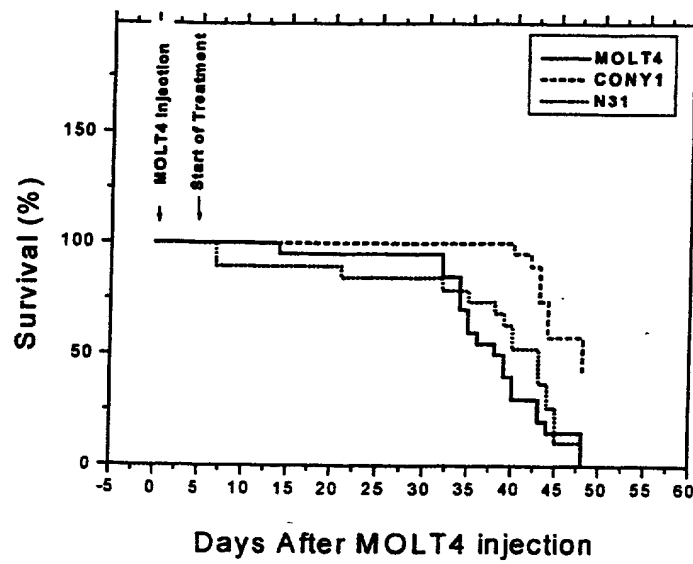
**FIG. 33**



**FIG. 34**

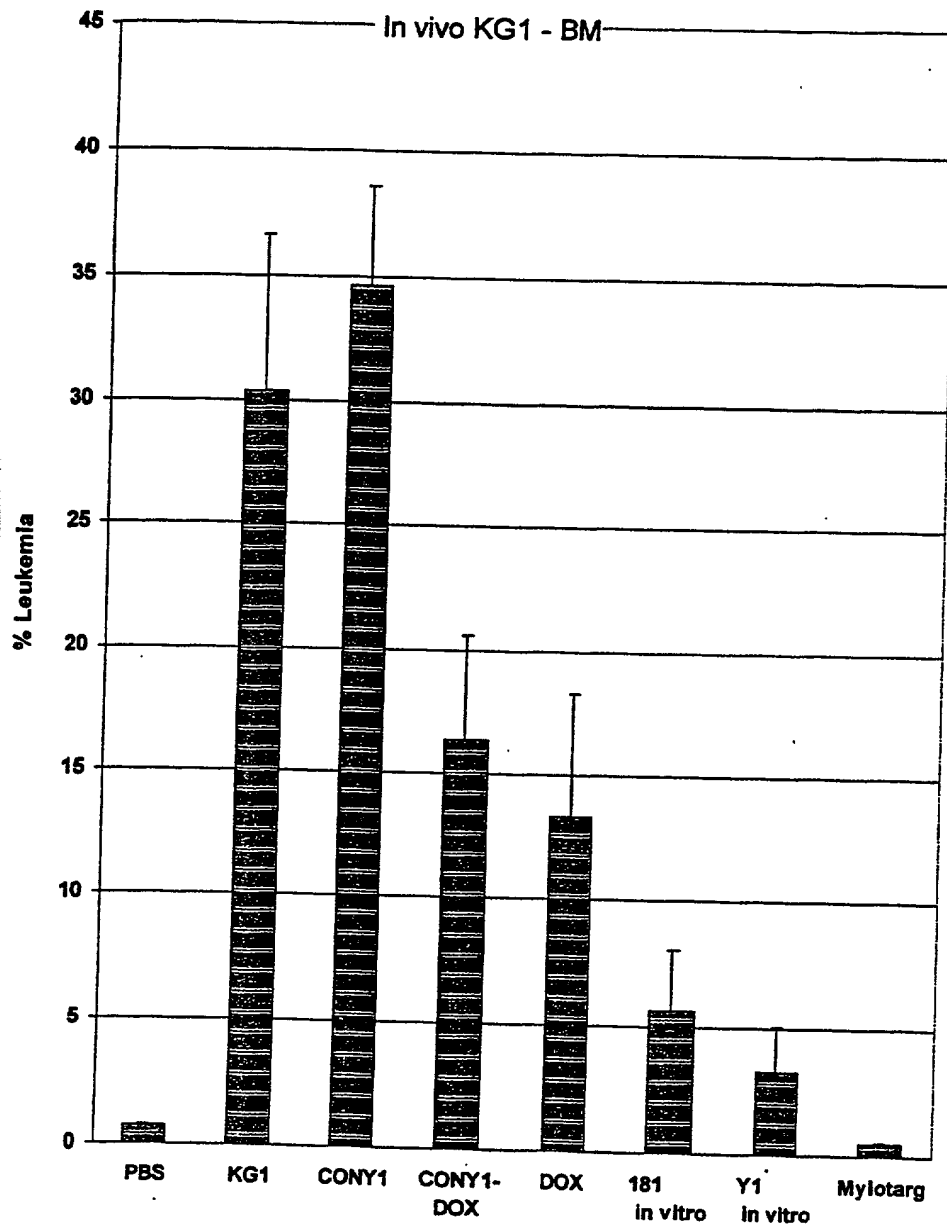


**FIG. 35**

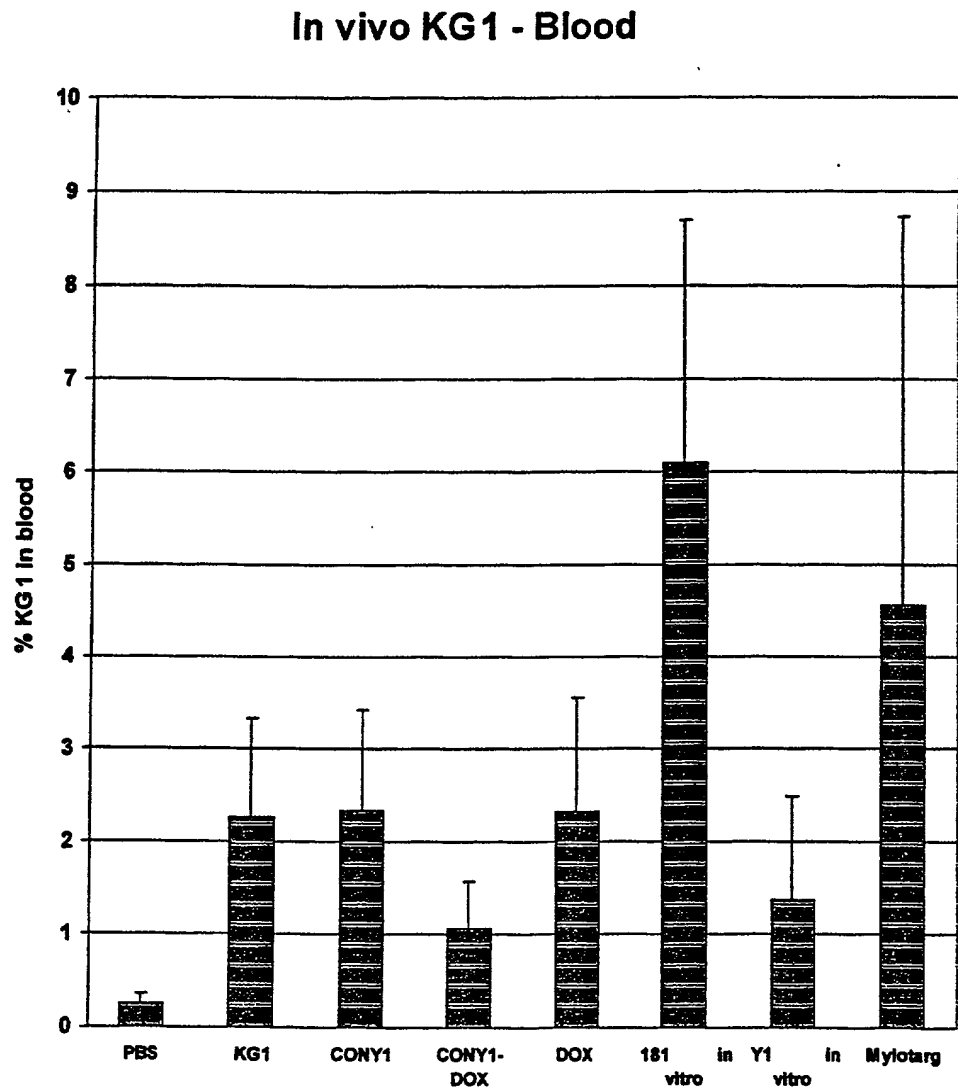


**FIG. 36**

\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.

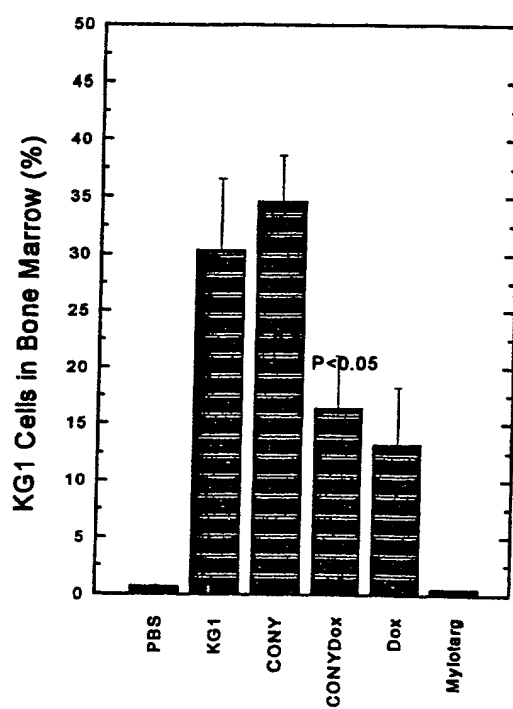


**FIG. 37**

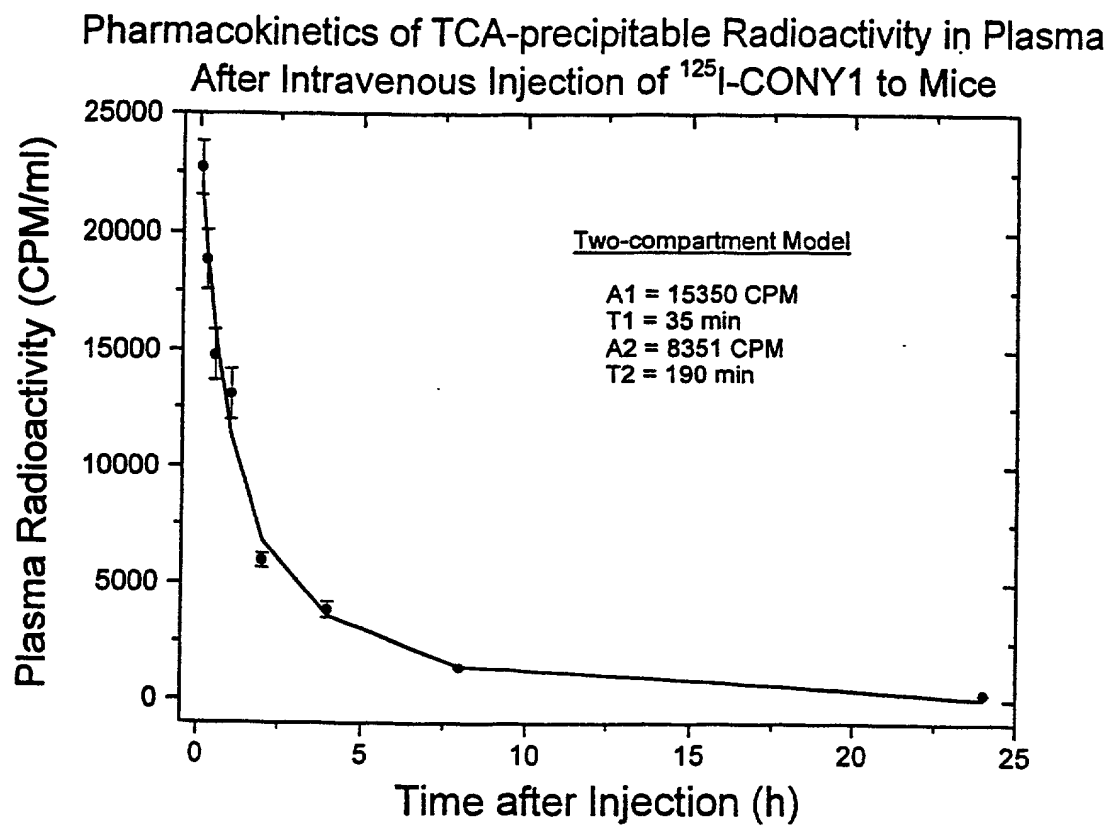


\*\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

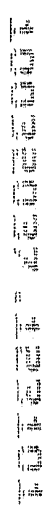
**FIG. 38**



**FIG. 39**

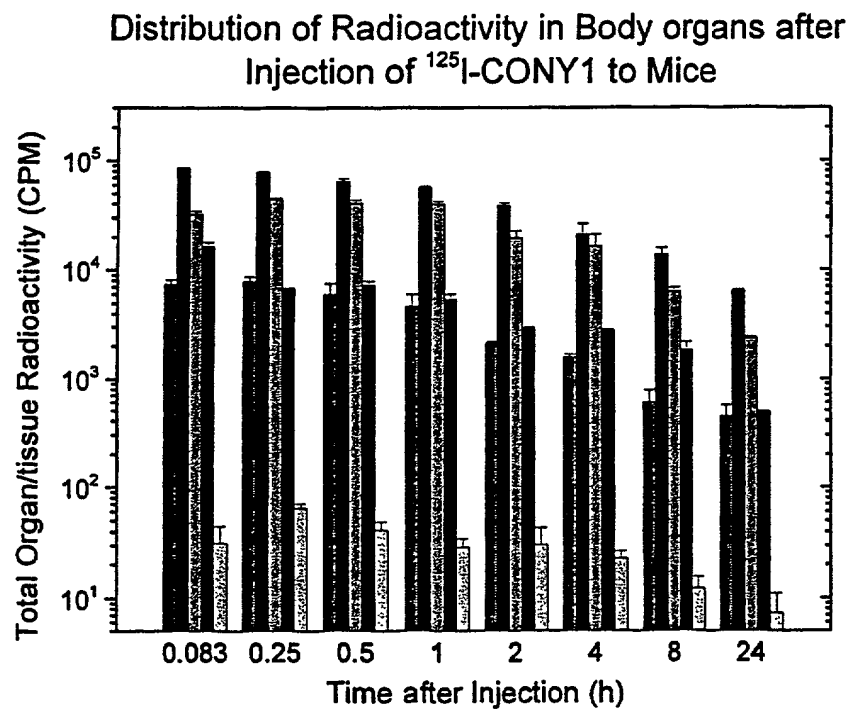


項目	単位	数量	備考
1. 材料費	円	100.00	
2. 労務費	円	50.00	
3. 経費	円	10.00	
4. 利益	円	20.00	
5. 合計	円	180.00	

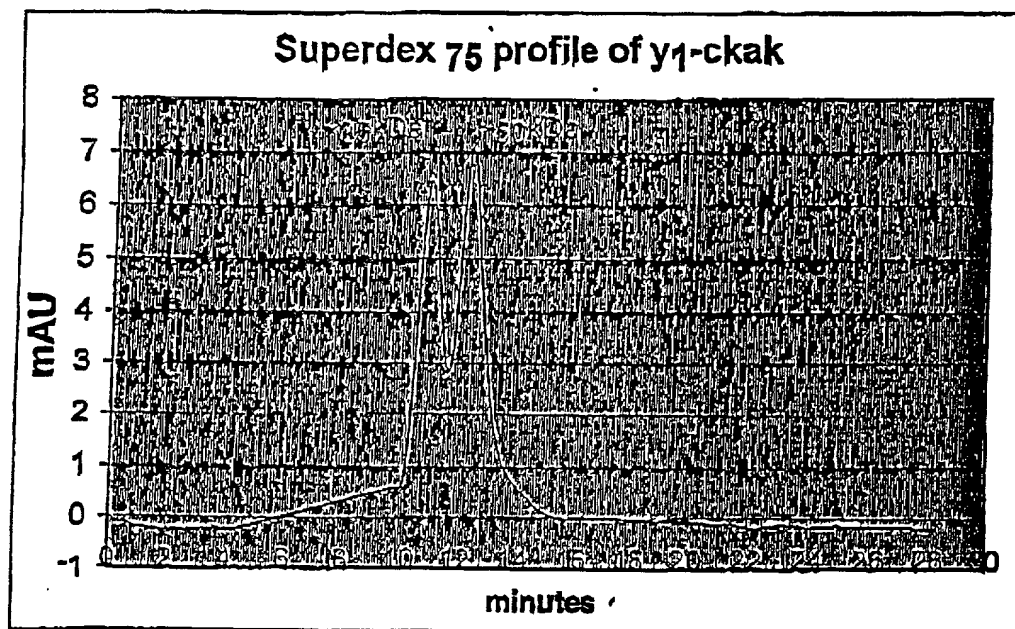




**FIG. 41**



**FIG. 42**



**FIG. 43**

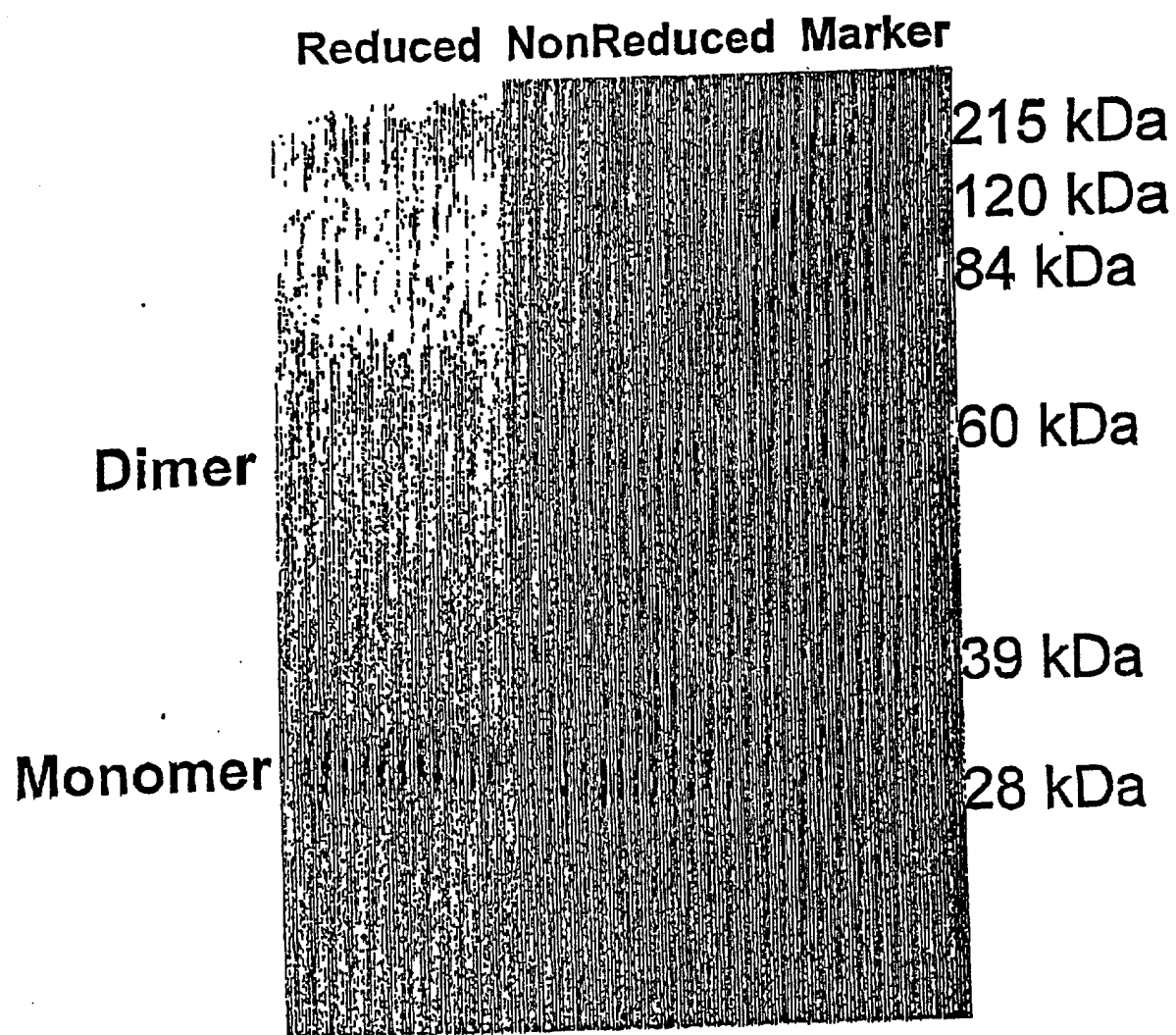
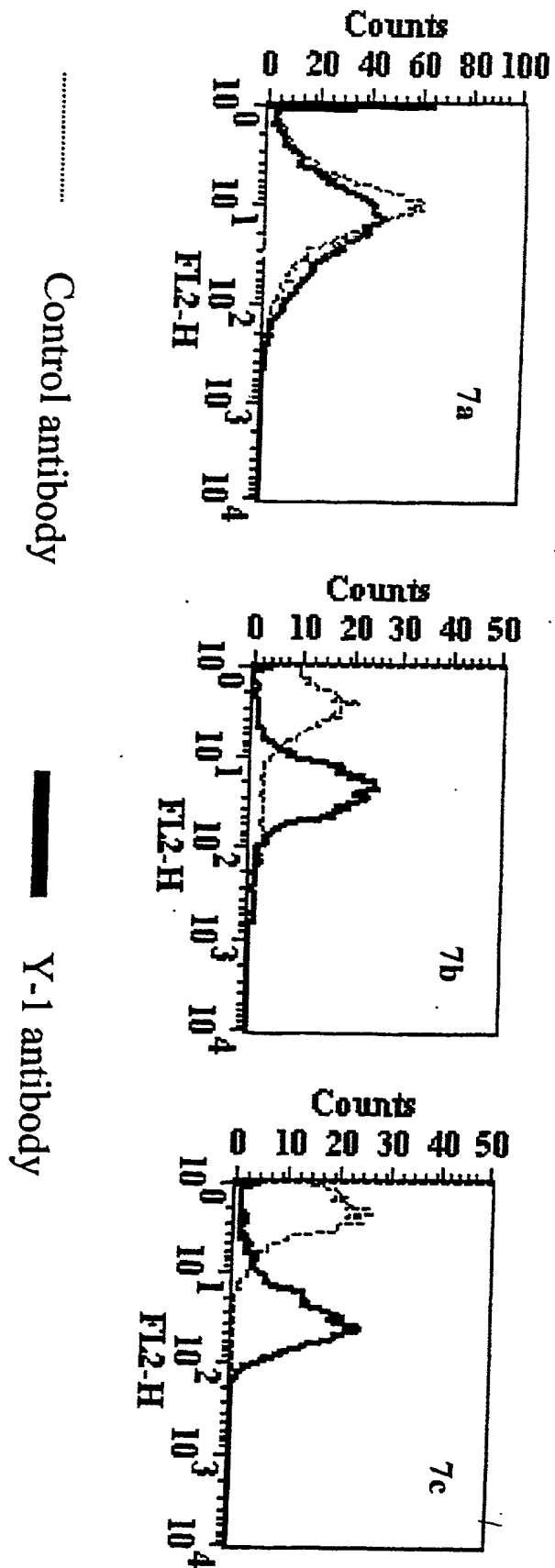
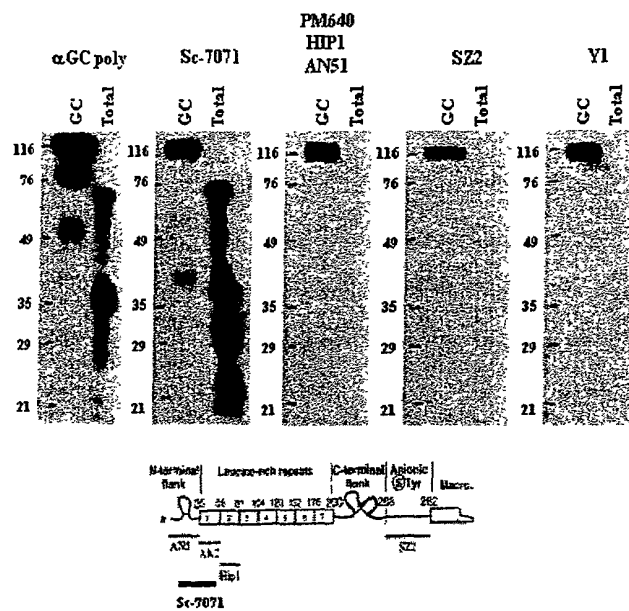


FIG. 44

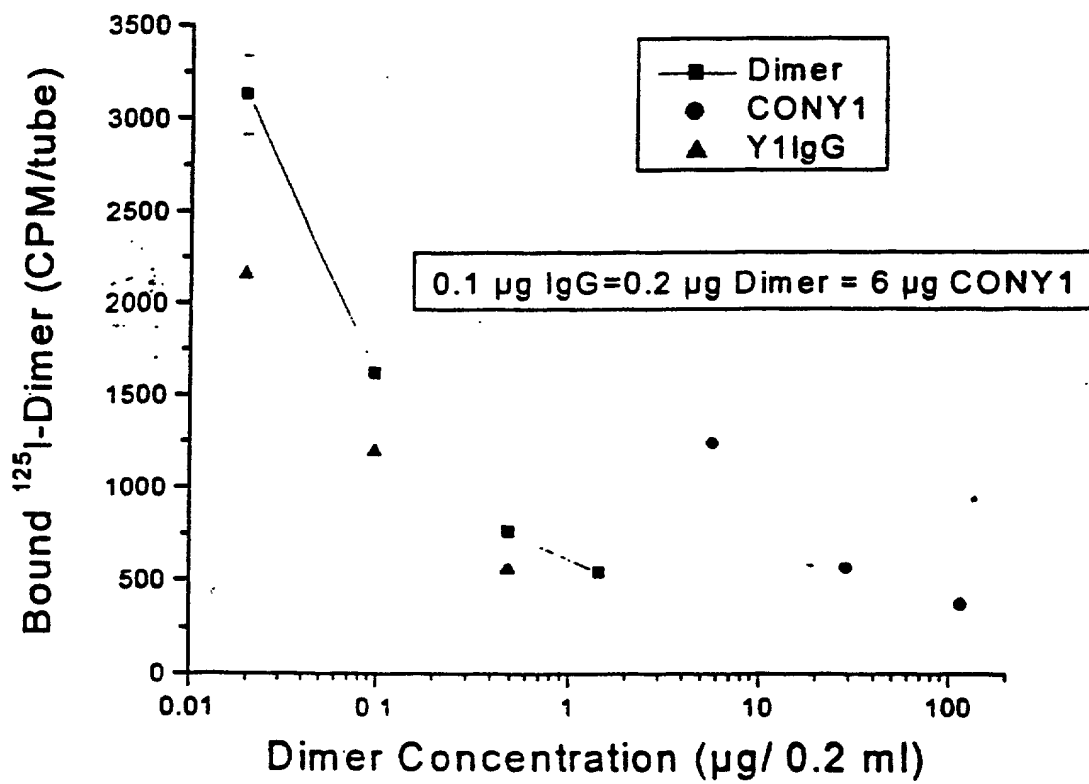


# FIG. 45

## Epitopes of anti-GPIb $\alpha$ antibodies



**FIG. 46**



**FIG. 47**

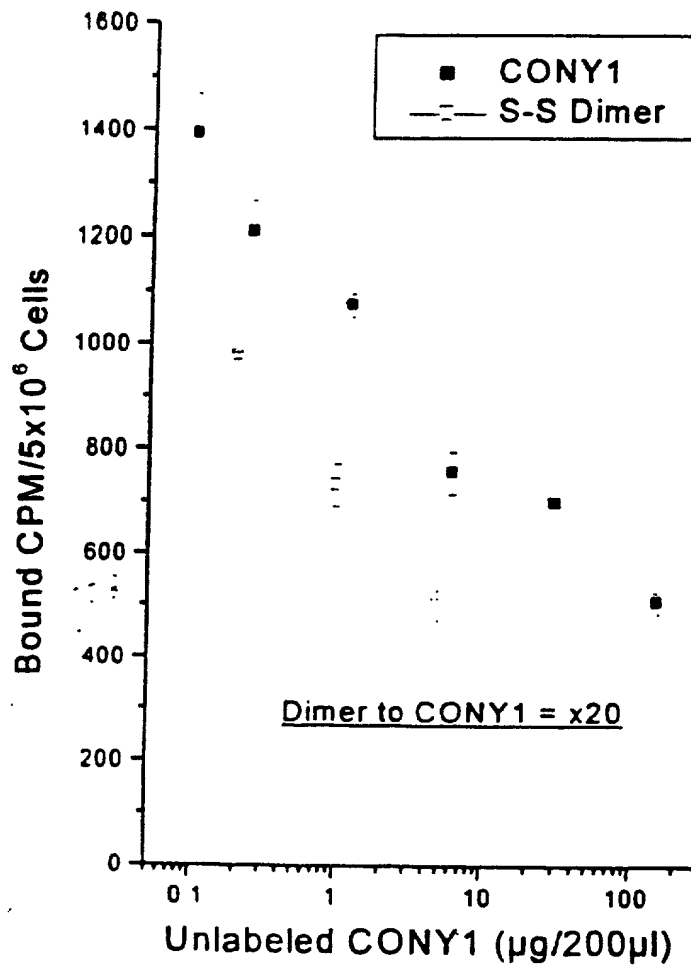


FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence); SEQ ID NO: 206 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	ATCCAGCTGCTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21	I Q L V E S G G G V V R P G G S L R L S
121	TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41	C A A S G F T F D D Y G M S W V R Q A P
181	GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61	G K G L E W V S G I N W N G G S T G Y A
241	GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81	D S V K G R F T I S R D N A K N S L Y L
301	CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101	Q M N S L R A E D T A V Y Y C A R M R A
361	CCTGTGATTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121	P V I W G Q G T L V T V S S A S T K G P
421	TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141	S V F P L A P S S K S T S G G T A A L G
481	TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTG
161	C L V K D Y F P E P V T V S W N S G A L
541	ACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC
181	T S G V H T F P A V L Q S S G L Y S L S
601	AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201	S V V T V P S S S L G T Q T Y I C N V N
661	CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAC
221	H K P S N T K V D K R V E P K S C D K T
721	CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACTGTGAGTCTTCOTCTTC
241	H T C P P C P A P E L L G G P S V F L F
781	CCCCCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261	P P K P K D T L M I S R T P E V T C V V
841	GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281	V D V S H E D P E V K F N W Y V D G V E
901	GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301	V H N A K T K P R E E Q Y N S T Y R V V
961	AGCGTCTCTACCGTCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321	S V L T V L H Q D W L N G K E Y K C K V
1021	TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCC
341	S N K A L P A P I E K T I S K A K G Q P
1081	OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGAGGAGATGACCAAGAACCAGGTC
361	R E P Q V Y T L P P S R E E M T K N Q V
1141	AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381	S L T C L V K G F Y P S D I A V E W E S
1201	AATGGGCAGCCGAGACAACACTACAAGACCACGTCTCCCGTGCTGGACTCCGACGGCTCC
401	N G Q P E N N Y K T T S P V L D S D G S
1261	TTCTTCCTCTATAGCAAGCTCACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421	F F L Y S K L T V D K S R W Q Q G N V F
1321	TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441	S C S V M H E A L H N H Y T Q K S L S L
1381	TCTCTGGGTAAATGA
461	S L G K *



**FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC**

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

```
1      ATGGCCTGGGCTCTGCTGCTCCTCACCCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1      M A W A L L L L T L L T Q D T G S W A D
61     GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21     A E L T Q D P A V S V A L G Q T V R I T
1212   TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41     C Q G D S L R S Y Y A S W Y Q Q K P G Q
181    GCCCCTGTACTTGTCTATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTG
161    A P V L V I Y G K N N R P S G I P D R F
241    TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81     S G S S S G N T A S L T I T G A Q A E D
301    GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGAACCATGTGGTATTCGGCGGA
101    E A D Y Y C N S R D S S G N H V V F G G
361    GGGACCAAGCTGACCGTCCTAGGTCTAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121    G T K L T V L G Q P K A A P S V T L F P
421    CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141    P S S E E L Q A N K A T L V C L I S D F
481    TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161    Y P G A V T V A W K A D S S P V K A G V
541    GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181    E T T T P S K Q S N N K Y A A S S Y L S
601    CTGACGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTACGCATGAAGGG
201    L T P E Q W K S H K S Y S C Q V T H E G
661    AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221    S T V E K T V A P T E C S *
```

# FIG. 49

	1	11	21	31	41	51	
1	EVOLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY	60
61	ADSVKGRETI	SRDNSKNTLY	LOMNSLRAED	TAVYYCARYA	KTLMRQYSLW	GQGTLLTVSR	120
121	GGGSGGGGS	GGGGSSELTQ	DPAVSVALGQ	TVRITCQGDS	LASYIASWYQ	QKPGQAPVLV	180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT	240
241	VLGAAAEQKL	ISEEDLNGAA					

# FIG. 50

		10	20	30	40	50	60
1	3	ATTATTACTc gCGGCCcAGC CgGCCcAGC CGAGGTGCAG CTGGTGGAGT CTGGGGGAGG					
		L L L A A Q P A M A E V Q L V E S G G G					
		70	80	90	100	110	120
1	3	CTTGGTACAG CCTGGGGGGT CCTGAGACT CTCCTGTGCA GCCTCTGGAT TCACCTTTAG					
		L V Q P G G S L R L S C A A S G F T F S					
		130	140	150	160	170	180
1	3	CAGCTATGCC ATGAGCTGGG TCCGCCAGGC TCCAGGGAAG GGGCTGGAGT GGGTCTCAGC					
		S Y A M S W V R Q A P G K G L E W V S A					
		190	200	210	220	230	240
1	3	TATTAGTGGT AGTGGTGGTA GCACATACTA CGCAGACTCC GTGAAGGGCC GGTTCACCAT					
		I S G S G G S T Y Y A D S V K G R F T I					
		250	260	270	280	290	300
1	3	CTCCAGAGAC AATTCCAAGA ACACGCTGTA TCTGCAAATG AACAGCCTGA GAGCCGAGGA					
		S R D N S K N T L Y L Q M N S L R A E D					
		310	320	330	340	350	360
1	3	CACGGCCGTG TATTACTGTG CAAGACCGG TCAGATTTTAAAGCGTAAT GGGGCCAAGG					
		T A V Y Y C A R T G Q S I K R S W G Q G					
		370	380	390	400	410	420
1	3	TACCCTGGTC ACCGTGTGCA GAGGTGGAGG CGGTTCAGGC GGAGGTGGCT CTGGCGGTGG					
		T L V T V S R G G G G S G G G G S G G G					
		430	440	450	460	470	480
1	3	CGGATCGTCT GAGCTGACTC AGGACCCTGC TGTGTCTGTG GcCTTGGGAC AgACAGTCAG					
		G S S E L T Q D P A V S V A L G Q T V R					
		490	500	510	520	530	540
1	3	GATcACATGC CAAGGagACA GCCTCAGAAG CTATTATGCA AGCTGGTACC AGCAGAAGCC					
		I T C Q G D S L R S Y Y A S W Y Q Q K P					
		550	560	570	580	590	600
1	3	AGGACAGGCC CCTGTACTTG TCATCTATGG TAAAAACAAC CGGCCCTCAG GGATCCCAGA					
		G Q A P V L V I Y G K N N R P S G I P D					
		610	620	630	640	650	660
1	3	CCGATTCTCT GGCTCCAGCT CAGGAAACAC AGCTTCCTTG ACCATCACTG GGGCTCAGGC					
		R F S G S S S G N T A S L T I T G A Q A					
		670	680	690	700	710	720
1	3	GGAAGATGAG GCTGACTATT ACTGTAAC TC CGGGACAGC AGTGGTAACC ATGTGGTATT					
		E D E A D Y Y C N S R D S S G N H V V F					
		730	740	750	760	770	780
1	3	CGGCGGAGGG ACCAAGCTGA CCGTCCTAGG TGCGGCCGCA GAACAAAAAC TCATCTCAGA					
		G G G T K L T V L G A A A E Q K L I S E					
		790	800	810	820	830	840
1	3	AGAgGatCTG AatGGGGCCG CAAGGAACTG TtGAATTTT TAAGTTAAcC T					
		E D L N G A A * N C * I F * V N					

Y16 SEQ ID NO: 210

## ***FIG. 51***

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL  
81 YLQMNSLRAE DTA VYYCARM RAPVIWGQGT LTVSRGGGG  
121 SGGGGSGGGG SSEL TQDPAV SVALGQTVRI TCQGDSLRSY  
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA  
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG  
241 GGLNDIFEAQ KIEWHE

## ***FIG. 52***

Y1-cys-kak scFv (SEQ ID NO. 212)

1 MEVQLVESGG GVVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM  
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY  
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR  
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK

Y1-cys-kak scFv (SEQ ID NO. 212)